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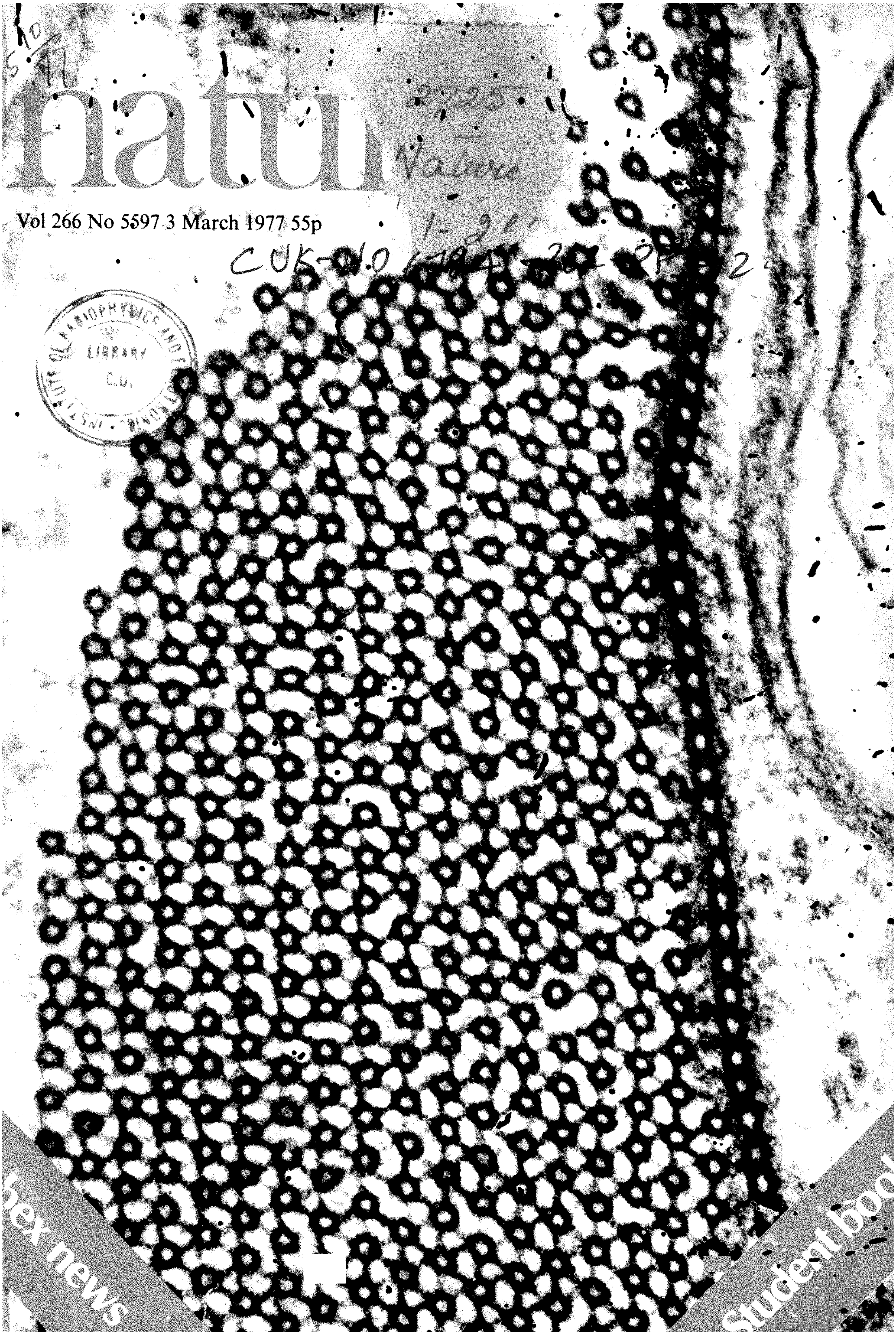

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A cytopharyngeal nemadesma in the ciliate *Nassula*—the assembly of microtubule bundles including nemadesmata is discussed on page 22.

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An infrared mosaic of Italy seen from LANDSAT-1. To obtain cloud-free pictures, photography was done in all four seasons.

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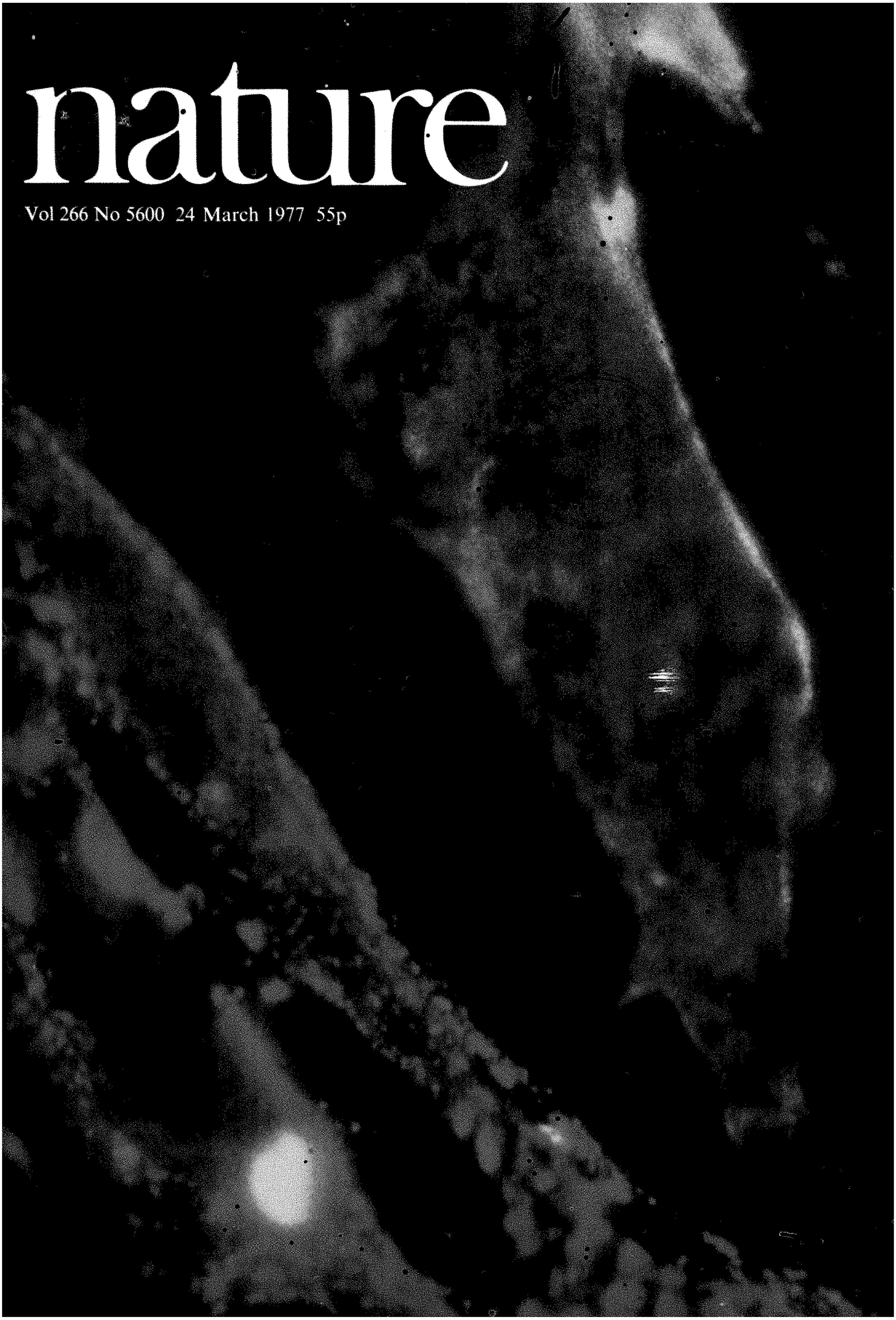

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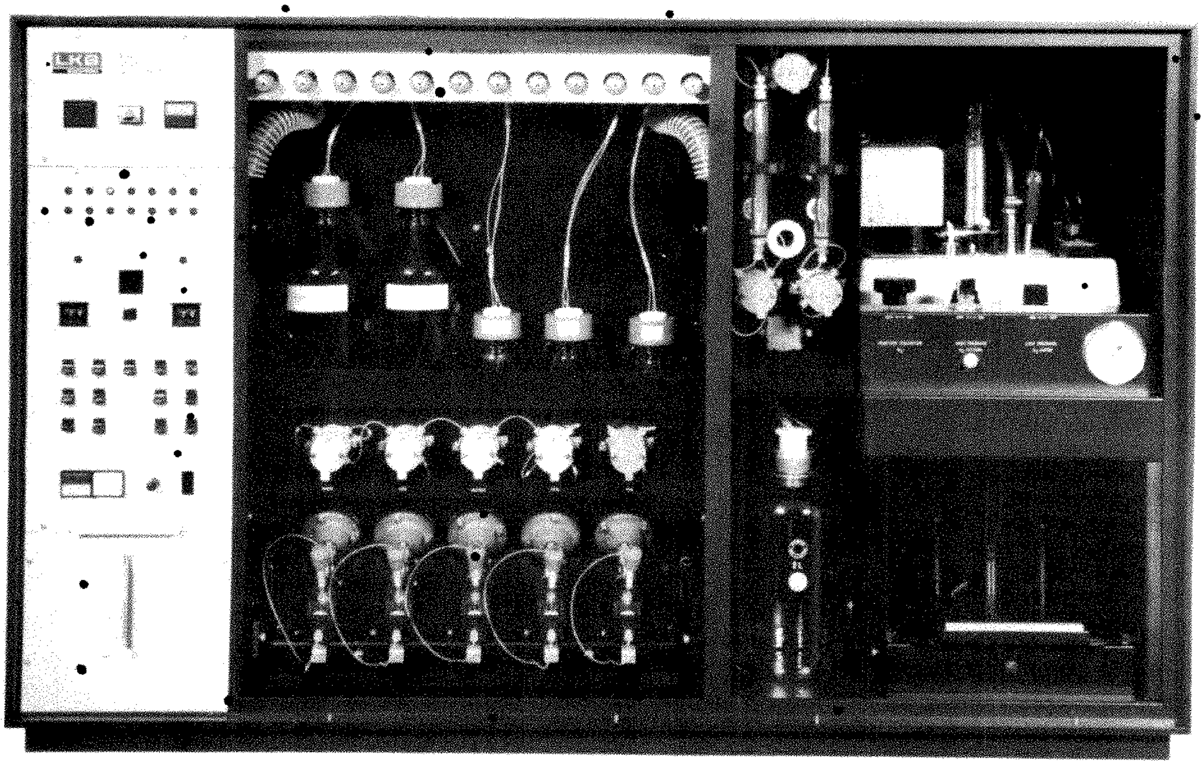

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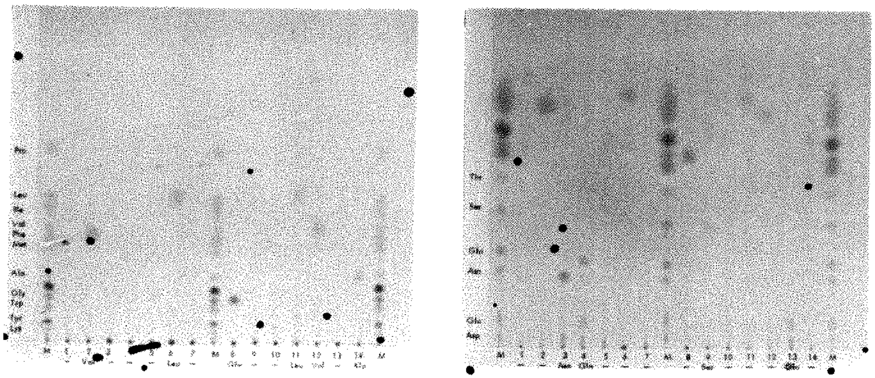

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Schwann cells (red) and fibroblasts (green) identified by fluorescent antibodies in dissociated cultures of rat nerve (see page 364).

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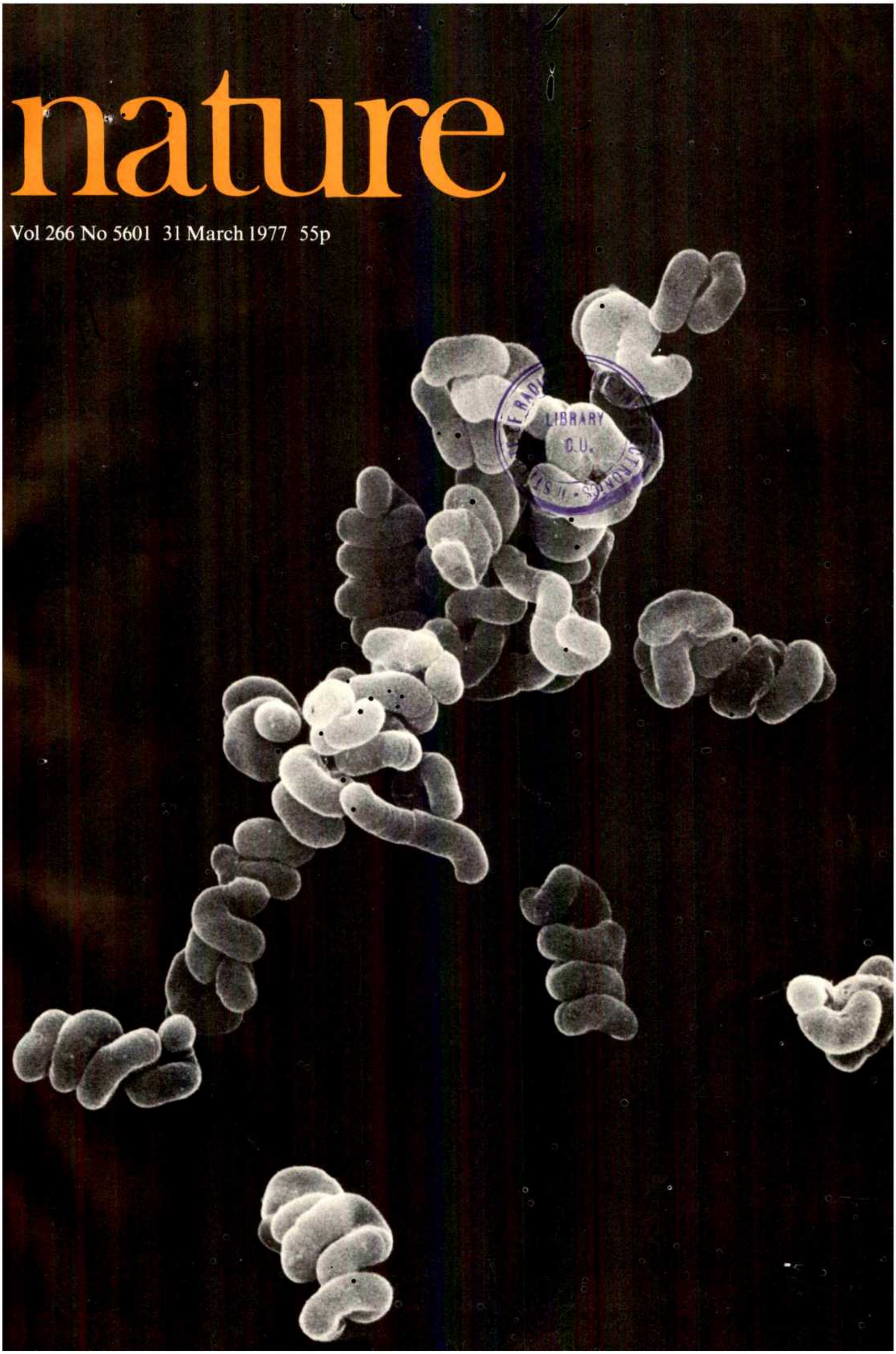

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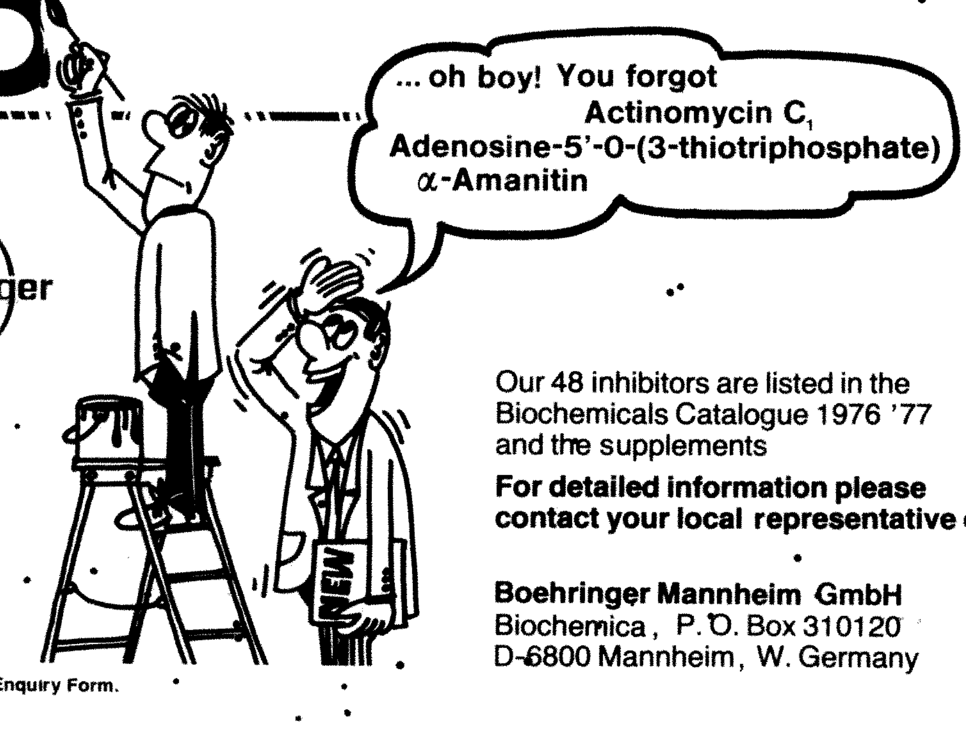
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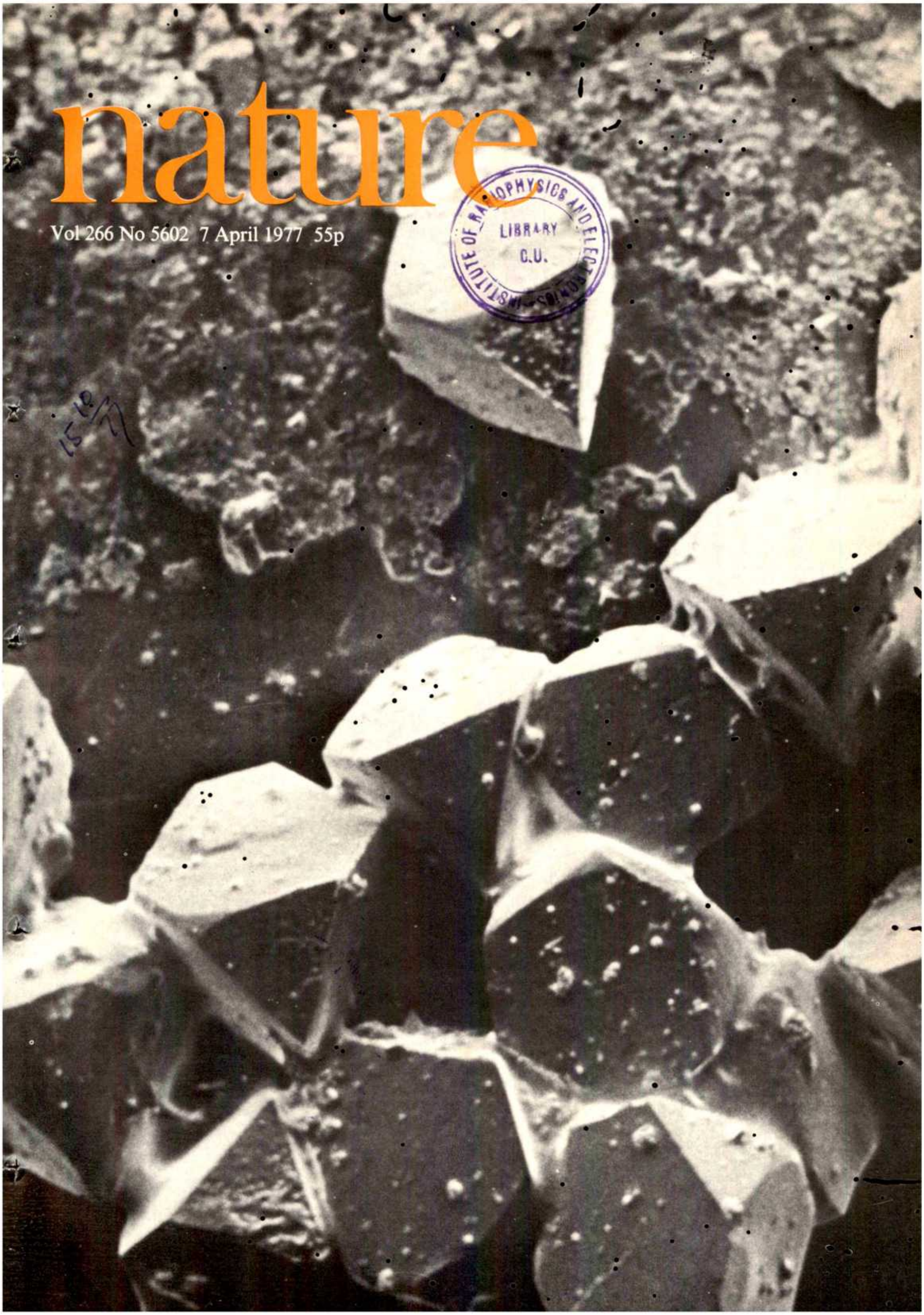

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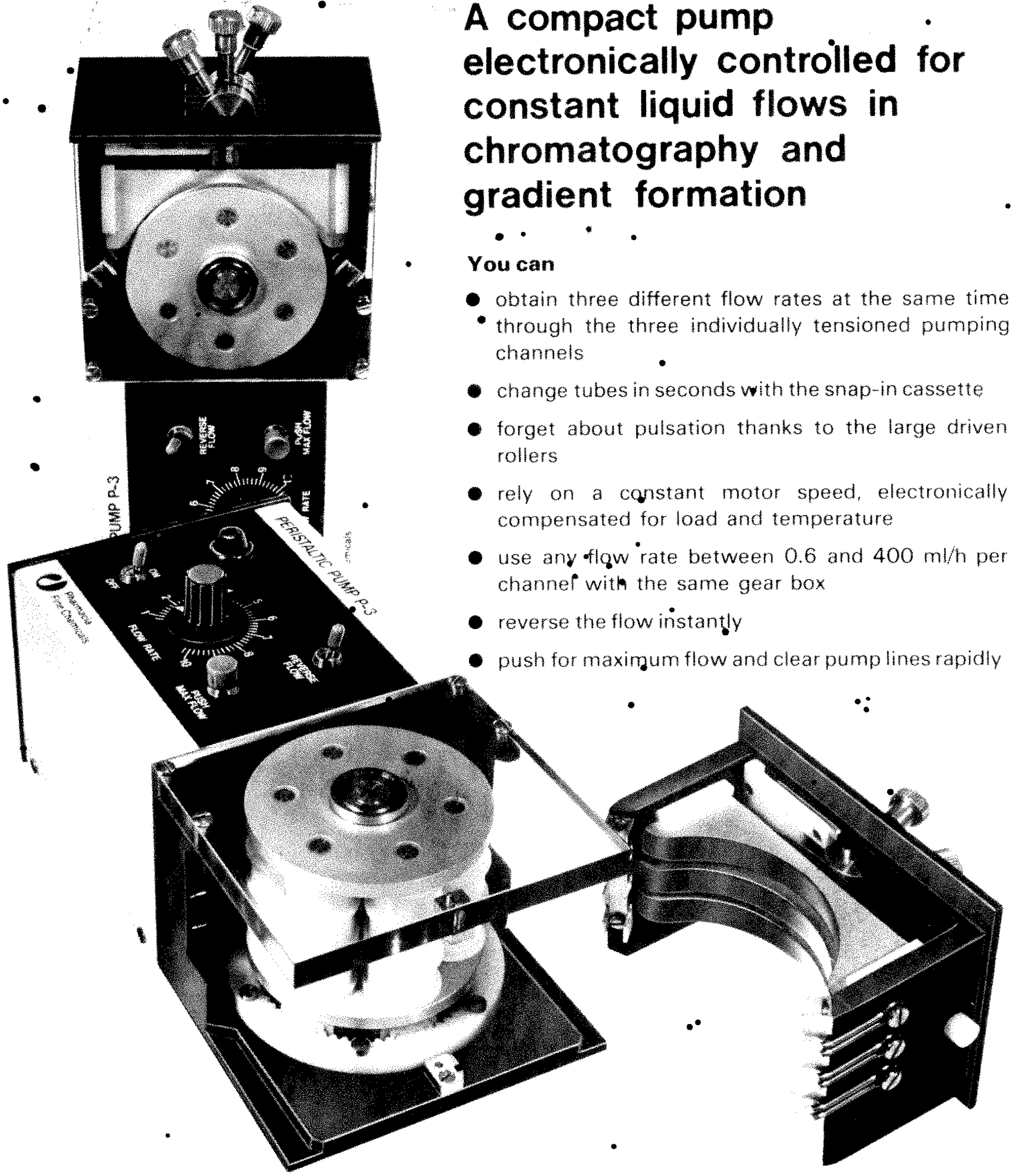

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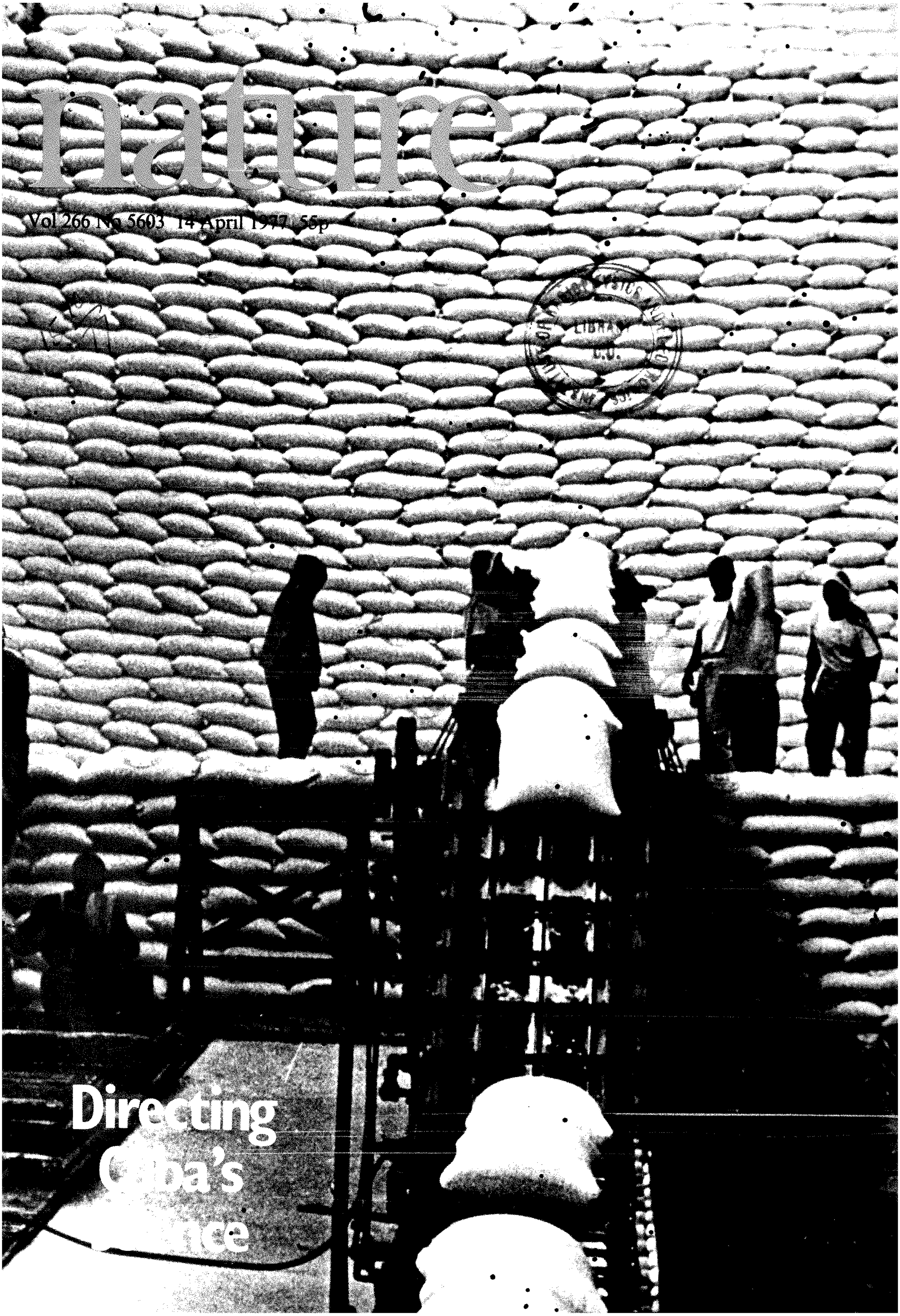

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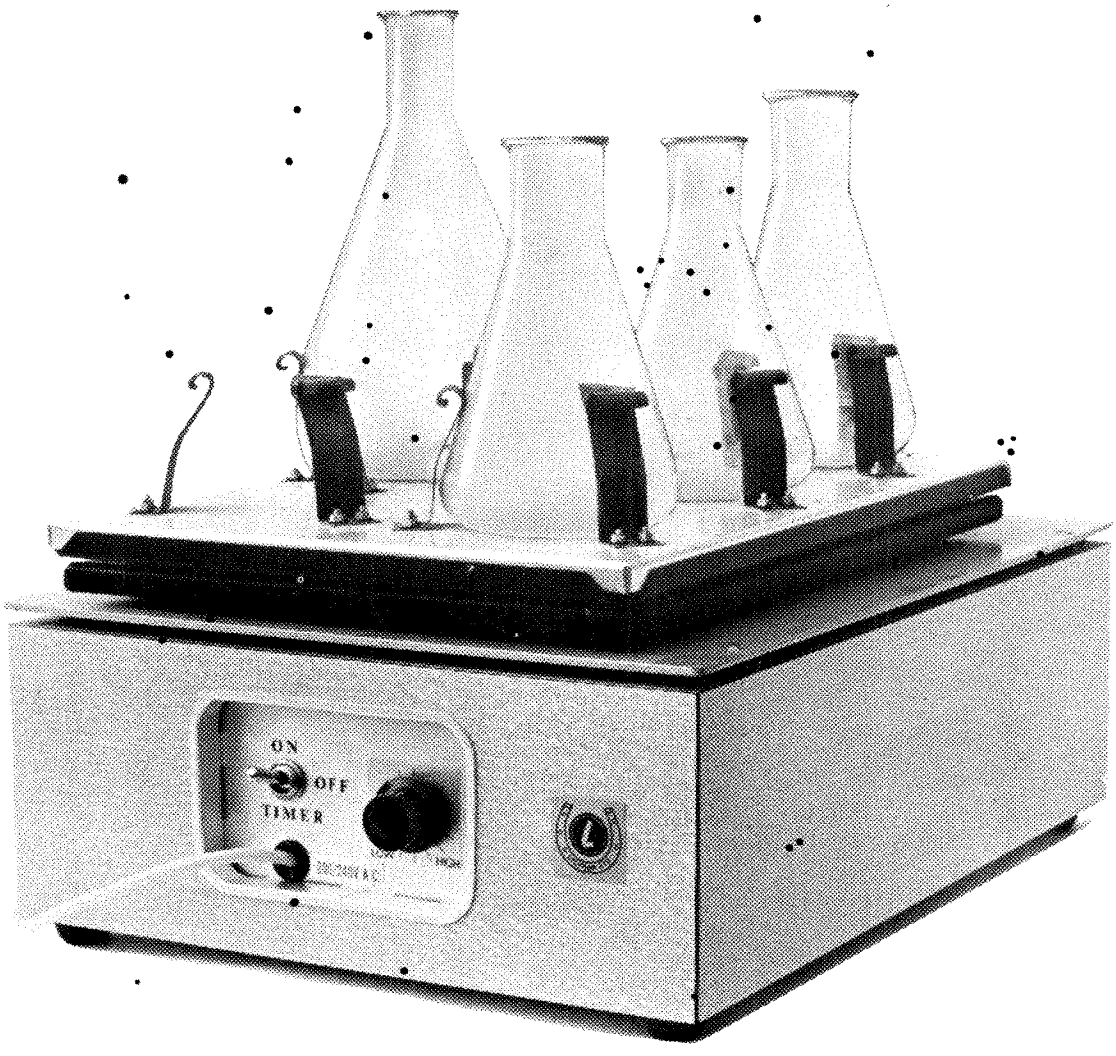

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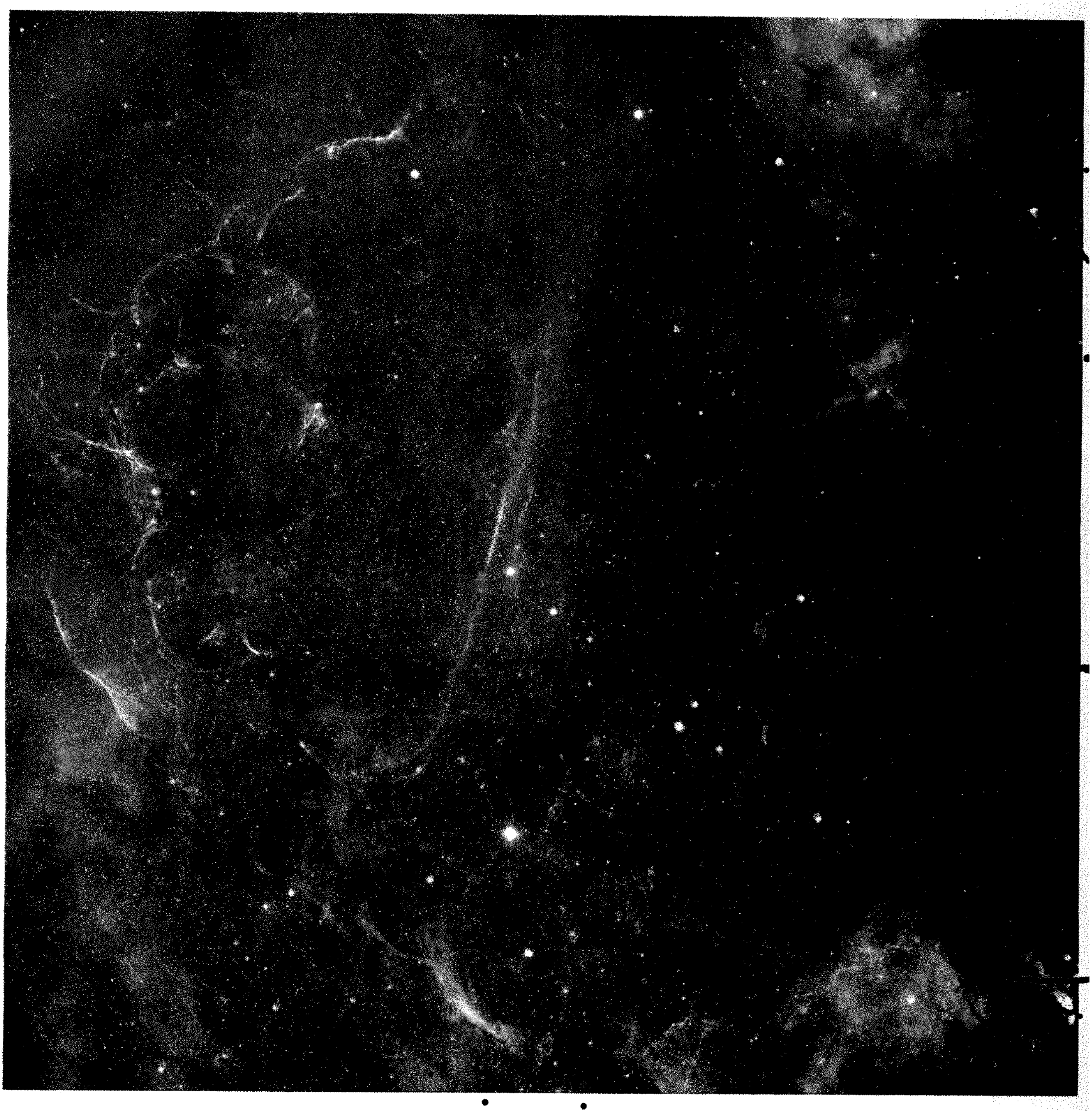

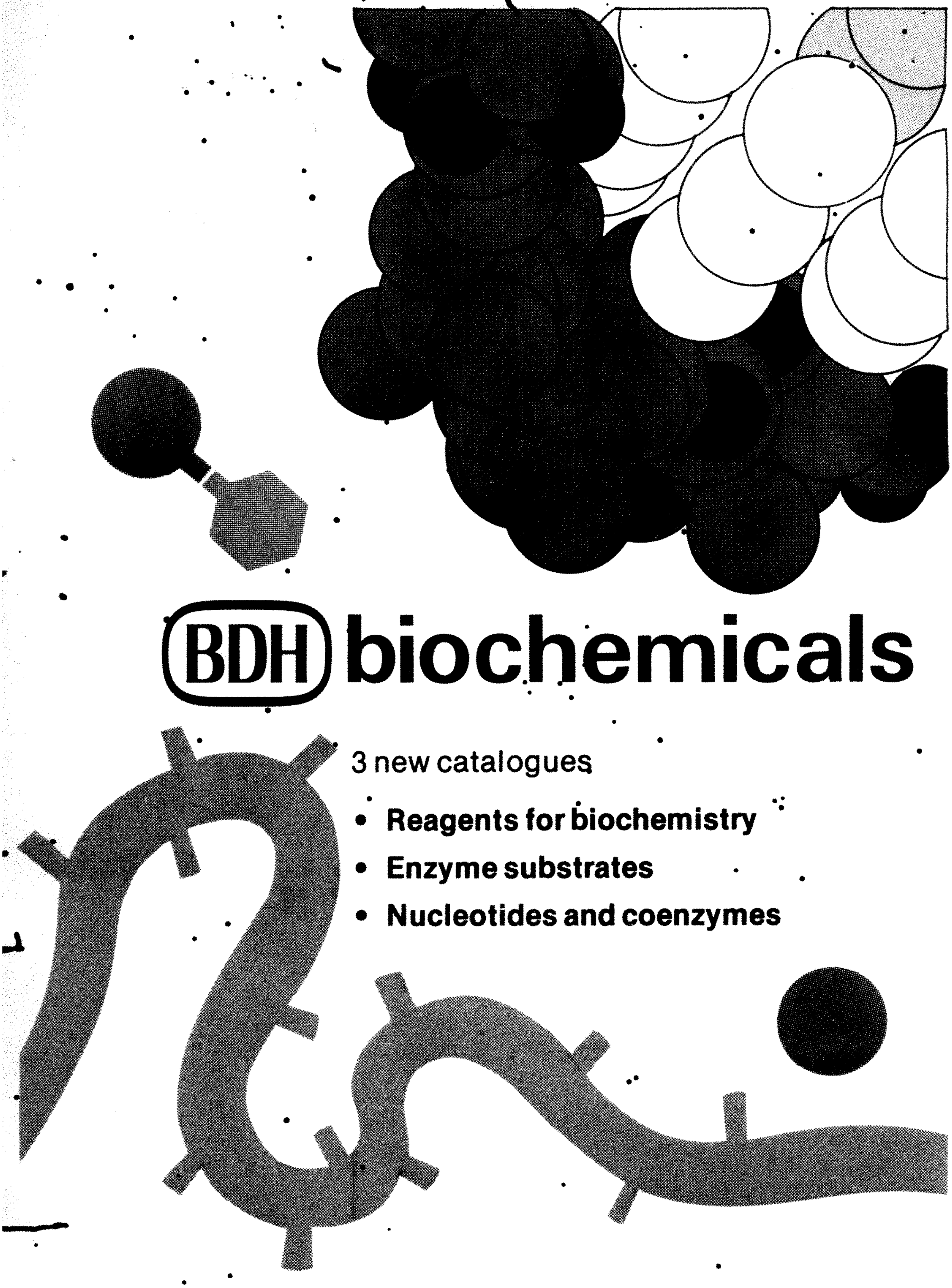

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A photograph (6½° × 6½°) of the Vela Supernova remnant, taken on the U.K. Schmidt Telescope at Siding Spring. A mosaic Hα filter, designed by John Meaburn, was used.

Optical pulses from the Vela pulsar are reported on page 692. See also page 676.

(Picture by courtesy of Royal Observatory Edinburgh)

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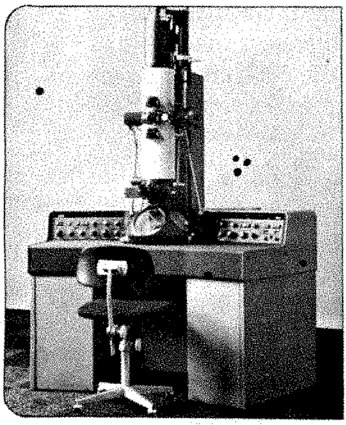

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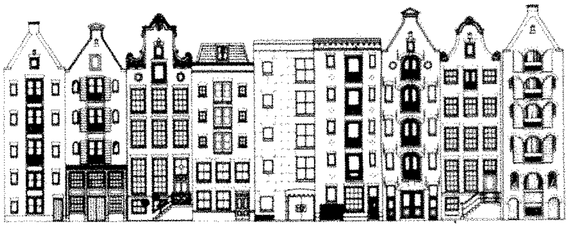

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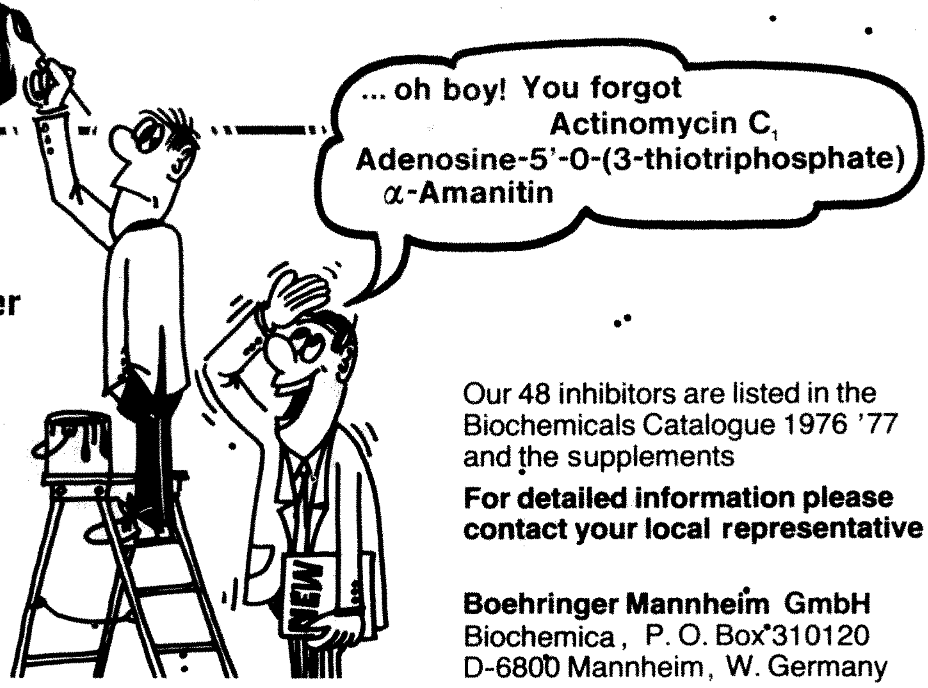

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nature

3 March 1977

The law moves in

A MEETING of the American Association for the Advancement of Science (AAAS) is a hard time for journalists. Somehow one has to find a theme behind it all; not just the anonymous title adopted for this year's gathering in Denver —'Science and Change: Hopes and Dilemmas'—but some ground base on top of which runs the counterpoint of corridor and coffee-shop conversation.

It isn't an easy task. First, there is the preposterous proliferation of sessions about which almost everyone seems to complain. At any one time you have to choose amongst weather modification, Mars, everyday physics, solar energy, energy in the Rockies, polar research, food pests, desert dust, behavioural research, organ transplants, early experience in primates, appropriate technology, cognition, minorities in science, limits to growth, information policy, religious minorities, systems research and the problems of a science academy. Second, scientists tend (as at the British Association) to drop in, cast their pearls and drop out. With some honourable exceptions this leaves little time for long conversations. Third, the rhetoric doesn't help— what do administrators really mean when they talk about addressing the issues through a hopefully ongoing soft-failure mode of evaluation, and assessment within a prioritised problem-solving environment?

And yet there was at least one theme to emerge from it all, and that was the increasing attention that lawyers are paying to the scientific and technological scene. Some of this attention has been in the field of human rights, where the remarkable action of President Carter in writing to Dr Sakharov has raised many hopes amongst both scientists and lawyers. But scientists and lawyers don't always find themselves fighting on the same sides, and several sessions were devoted to the difficult issues of scientific freedom and responsibility, not just in the abstract but with pressing matters right to hand. The Governor of Colorado, Richard Lamm, who came to power not by the conventional political route but through an environmental concern which captured the state's imagination, put the problem remarkably succinctly in a public lecture. He had, he remarked, been surprised to see a session entitled 'Science: the Key to our Political Future'. (He would have been even more surprised if he had attended the session by some of the let-the-scientists-get-on-with-it views expressed.) For Governor Lamm a more appropriate title would have been 'Politics: the Key to our Scientific Future'. And the theme that science and technology must expect to be increasingly circumscribed by politicians, the courts and public opinion was widely taken up.

It is not only the recombinant DNA question which has aroused some new concerns. The legal position of those working on earthquake prediction is likely to be questioned before long in the courts. The recent Teton Dam disaster in Idaho, subject of an excellent documentary shown, coincidentally, on television during the conference, raises further questions of how much responsibility the scientist can bear for a project that fails. But the most striking example at present was on the AAAS's doorstep in Colorado —weather modification. The western United States is in the grips of a drought and there is little snow on the mountains to provide relief this spring. The Denver Hilton was doing its bit, it announced, by not providing iced water for customers in its restaurants unless they specifically asked for it. A golden opportunity for rain-makers to show their simple yet still highly debated skills. One such person boldly distributed his advertisements around the conference, 'for rain in only 6 days, anywhere, call . . . '. But things are becoming less simple. A whole day was devoted to a scientists/lawyers meeting on the law of weather modification, and the very same day the newspapers announced that if Washington state starts cloud-seeding, its neighbour, Idaho, will go to law to stop it.

There is little doubt that the law in the United States contains many as yet unexplored pitfalls for the scientist. There is a crucial distinction between speech and action in the constitution, which projects (in the most lurid example) the man who curses the flag but not the man who burns it. Likewise scientists seem to have the right to write, say and publish fairly freely, but what they do has no similar protection. The opposition to scientists is bound to grow— one honoured alumna of the University of Colorado, Boulder, told the local newspaper, 'I had a fairly competent position in science and felt impotent to do anything about the directions it was taking. As an attorney, I have a much better shot at controlling what they're doing than I had on the inside'. Scientists had better start facing these questions —not, one hopes, from a guild mentality, nor from the exaggerated stance heard at the conference that all science is being threatened, but simply because a good working relationship with lawyers is going to be necessary for science to flourish in the future.

Several people commented almost nostalgically that AAAS had been the quietest for years. No tomatoes thrown, no bitter squabbling, no police being called in. These days the police have been replaced by lawyers. □

Heading for harmony?

The debate prompted by genetic manipulation developments is intensifying in Europe. **Chris Sherwell** reports

IN PARIS on Tuesday a committee gathered quietly for its routine monthly meeting to discuss research involving genetic manipulation using recombinant DNA technology. In Brussels last Thursday another committee lodged deep in the labyrinth of the European Commission of the EEC met to discuss the same subject. On Thursday of this week Britain's equivalent body gathers for its own regular monthly session. Within the next week a commission on genetic manipulation in the Netherlands is expected to report its long-awaited findings. And on 15 March a new and potentially crucial group within the European Science Foundation (ESF) convenes its first meeting in Strasbourg.

Plainly, the pace of developments relating to recombinant DNA research in Europe has quickened. The debate is now consuming the time of government officials, science administrators and molecular biologists in at least nine different countries and no less than five international organisations. Indeed, the aforementioned meetings are but the tip of an enormous iceberg, and if activity is symptomatic of decisiveness, the energy now being spent discussing the subject ought inevitably to produce results. For those researchers actually wanting to do the work, however, it has meant long periods of delay, uncertainty and confusion.

The debate itself stems from the development over three years ago of elegant new genetic engineering techniques, the implications of which prompted widespread concern. This spawned the growth of guidelines, specifying conditions under which various types of experiment using the techniques should be done, and the growth of committees to administer them. The result is that the issue has spread well beyond the bounds of the research community immediately involved.

In the United States for example, where the pace has been hottest, the debates freest and public involvement greatest, the National Institutes of Health (NIH) finally published a complex set of guidelines last June. Failure to broaden these voluntary controls now threatens legislation to make them stick (see box), a development which could complicate the European debate.

So far the European debate has proceeded unsensationally, mostly at the national level; progress is varied. International discussions have gone quietly ahead, both informally among individuals and formally through those bodies which have perceived a role for themselves. The chances of them continuing without rancour could diminish, however, particularly if national differences emerge strongly in certain international forums.

Country catalogue

Most European countries have in fact trodden broadly similar paths. The examples of the United States and Britain have been important, the latter adding a new word to the English vocabulary: 'geemag'. The Genetic Manipulation Advisory Group (GMAG) was established in Britain last year following recommendations from the Williams working party on genetic manipulation.

Precautions contained in the Williams guidelines, like those in the NIH guidelines, involve both physical and biological containment. The standards of physical containment are more strict under Williams, however, while biological containment receives greater emphasis in the NIH guidelines. Among other differences, the NIH guidelines provide an extensive list of experiments and appropriate containment measures; under Williams GMAG decides matters case by case. GMAG itself had early problems over trade union representation, but later began business and by this week was meeting again (see box).

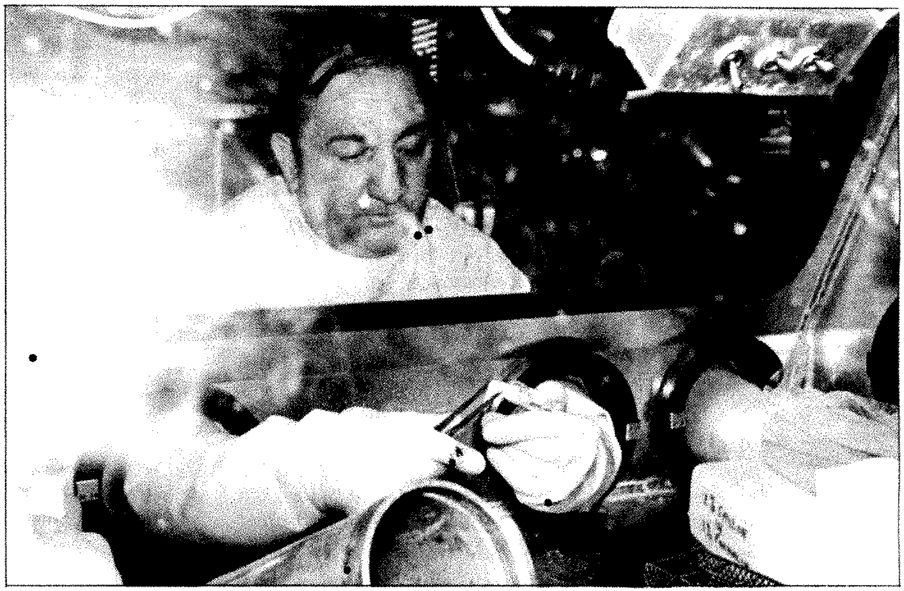
At least eight other countries in Europe have sought to involve themselves in the same sort of process.

France. In France two committees are directly involved. One, called an 'ethics committee' and having under 10 members, meets spasmodically to discuss the whole subject of genetic manipulation. The other, known as the *Commission de Controle*, was set up in 1975 under the auspices of France's main research body, the *Delegation Générale de la Recherche Scientifique et Technique* (DGRST). It has a membership of about a dozen, most of them with interests in recombinant DNA and related research. Industry and trade unions are not directly involved, though this could change; both are evidently prepared to abide by the *Commission* line.

The *Commission* meets regularly once a month to assess the conditions under which experiments in France are to be performed. It began by using the Asilomar guidelines, the product of the Asilomar conference in California in 1975, and subsequently used the NIH guidelines. More recently it has used both the Williams and NIH guidelines.

Over a period of some 18 months it has considered some 50 applications. Less than 10% have involved the more stringent containment conditions equivalent to the P3 and P4 categories of the NIH guidelines. The number of facilities in France which could now provide such containment is no more than a couple, and those would probably be at the P3 level.

Germany. The more fretful search for West German guidelines has produced four drafts, the latest of which was due to be distributed by the end of February, with the hope of producing a final draft by the spring. The government department involved, the Ministry of Research and Technology (BMFT), took over after a somewhat unsuccessful start under the German Research Council (DFG) using NIH guidelines.



Topix

Physical containment at Porton

The new national guidelines are expected to follow much the same lines as the NIH and Williams precedents. There will probably be an equivalent to Britain's GMAG with mixed representation. Guidelines will be voluntary, and it is hoped that industry, for the inclusion of which there are apparently no immediate plans, will follow them. There are no national P4 facilities in Germany, and few that would qualify as P3 facilities.

Switzerland. The Swiss Academy of Medical Sciences created a standing committee known as the Commission on Experimental Genetics. Headed by Werner Arber, Professor of Microbiology at the University of Basel, it has a membership of about a dozen, consisting of experts in the field and government officials from the health and science and technology ministries. Industry is ostensibly as keen as the universities to see controls implemented, and is represented on the Commission.

The Commission decided recently to follow the NIH guidelines, but this could still change in the future. It has been agreed with government agencies that no legislation is necessary. Researchers applying to the Swiss grant-giving body (*Suisse National Fonds*, SNF) and supplying relevant details would go before the Commission only when the SNF is unhappy; but they will probably be invited to register their work.

Sweden. An 11-man "committee concerning research with recombinant DNA" was set up last spring under the auspices of the Natural Science Research Council (NFR), the Medical Research Council (MFR) and the Swedish Cancer Society (RmC). Its chairman, Professor Peter Reichard of the Karolinska Institute, is the appointee on the committee of the NFR, which also appoints two lay representatives—in this case two MPs. The MFR, RmC, the Council for Forestry and Agricultural Research, the Board of Health and Welfare and the Association for the Pharmaceutical Industry each appoint one member; one member jointly represents the Academy of Sciences and the Academy of Engineering Sciences, another jointly represents the National Defence Research Institute and the Board for Technical Development; the Central Organisation for Salaried Employees appoints a representative for technical staff.

The committee will help granting agencies and government authorities to determine safety conditions for the experiments which they fund, and will probably work in the private sector as well. The committee will also advise researchers over safety precautions and help in risk classification, for which a working group has just been appointed. Researchers will be expected to submit experimental protocols to national advisory committees, which are responsible for specifying the containment measures. The containment procedures proposed are those of Williams, but experiments prohibited under the NIH guidelines, it is suggested, should not be carried out. Local safety committees would be responsible for supervising the measures.

So far the committee has received no applications and no recombinant DNA work is proceeding. The only group in Sweden which has worked in the field is at Uppsala under Professor Lennart Philipson, the MFR's appointee on the committee. His work is in abeyance, and he has yet to submit an application. The group has applied to have two new P3 laboratories built, but finance for these is not yet assured.

Denmark. Denmark has two committees. One, headed by Dr Kjeld Marcker from Aarhus, is an ad hoc committee established by the country's research council. It is examining work being done in Denmark and hopes to decide by the summer whether there ought to be a special research programme. The other is a Committee of Registration with 10 members representing research councils and industry but not trade unions. Its concern is with safety aspects, and it has been operating only a few months. The precise course it follows depends on the outcome of the first committee's work.

Holland. In January of last year, after consultation between the Royal Dutch Academy of Science and the National Health Council on the one hand, and the Ministry of Education and Science and the Ministry of Health on the other, a "Commission on Genetic Manipulation" was formed consisting of experts in the field and chaired by Professor Bootsma of Erasmus University, Rotterdam.

Its task was to compile an inventory of recombinant DNA research being done in the country and to advise laboratories on safeguards and the authorities on controls. The commission is expected to report its findings within the next two weeks, but there are differences of view about whether these should actually be published. The Dutch began by opting for the NIH guidelines but then switched to the Williams guidelines; now, however, it seems possible that national guidelines will be proposed which are more strict than either.

So far industry has not shown any public interest in the guidelines. Applications have come for six projects, from four universities. Three have come from the University of Amsterdam, and one from the Free University of Amsterdam, the University of Leiden and the University of Groningen.

Israel. A committee of the Israel Academy of Sciences and Humanities, chaired by Professor Leo Sachs of the Weizmann Institute, has recommended the establishment of a national safety committee for recombinant DNA research and of special safety committees at universities and research centres.

Legislation for US?

Legislation to control recombinant DNA research in the United States, a prospect which clearly concerns many scientists, is now considered inevitable. Bills have already been introduced into Congress, and in the next few weeks a committee consisting of officials from a number of federal agencies is expected to draft legislation to be submitted to Congress with the backing of the Carter Administration.

The Administration is reluctantly being pushed into drafting its own bill because if it doesn't, it will invite the prospect of harsher legislation and inconsistent local controls on recombinant DNA research. At present, the only formal controls on recombinant DNA research in the United States are guidelines issued last June by the National Institutes of Health (NIH), but they apply only to research supported by the federal government, and they are not backed by any enforcement or monitoring mechanism. Industry is not bound by any regulations.

Those apparent weaknesses in the present approach have prompted several state and local governments to consider adopting their own regulations on recombinant DNA research, and the fact that industrial research is not regulated at all has caused considerable public concern. Consequently, an inter-agency committee was established late last year under the chairmanship of NIH Director Donald Fredrickson to examine ways to extend the NIH guidelines to cover all recombinant DNA research in the United States. The committee has tentatively decided that no existing federal law can be used to extend and enforce the guidelines, and that new legislation is required.

That tentative conclusion was conveyed by Fredrickson last week to a group of prominent scientists and university administrators which met at NIH. Though Fredrickson repeatedly noted that no decision has been reached on the substance of the Administration's bill, it would almost certainly be designed simply to turn the NIH guidelines into enforceable regulations. It is also likely to include a clause pre-empting state and local regulations in an effort to ensure that regulations are uniform throughout the United States. It is not clear which agency would do the enforcing—clearly NIH wouldn't want to be in the position of supporting and regulating the research—but the likelihood is that the Center for Disease Control would be given the responsibility.

Colin Norman

The local committees would recommend appropriate safety precautions for every research proposal and then pass the matter to the national body, which has the final word.

The Academy decided that Israeli researchers would in general follow the NIH guidelines but would take into consideration recommendations from other bodies.

International catalogue

For Europe's researchers, worry as well as confusion is the product of the growing involvement of an increasing number of international bodies. But the important issue—whether or not there should be harmonisation of guidelines—already seems settled. There were benefits in allowing each country to follow its own path and arrive at its own conclusions, but different precautions in different countries threatened to encourage a concentration of researchers, so harmonisation looked preferable.

Moreover, with the subject a matter for public debate, there was the possibility that if one country applied more stringent controls than another, the difference itself could become an issue. Harmonisation could pose problems, though, if US researchers are faced with legally enforced regulations and there is public pressure for similar regulations in Europe. Creating a disinterested peer group competent to judge colleagues' work could be difficult enough without the added (if necessary) complication of drafting and enforcing legislation.

The case for harmonisation does not, however, solve the problem of which body has the authority to encourage the move towards it. A look at the organisations involved reveals the difficulty.

The World Health Organisation (WHO). The WHO has two bodies actively involved, the WHO Special Programme on Safety Measures in Microbiology and the WHO Environmental Programme. Being global and not confined to Europe, and concerned with the public health and safety aspects of the research, the WHO interest is peripheral as yet, although it has begun consultations, with, for example, Professor S. W. Glover, chairman of the International Microbial Genetics Commission, which has a genetic engineering sub-group.

The International Council of Scientific Unions (ICSU). ICSU has recently created a sub-committee known as COGENE, headed by the US microbiologist Bill Whelan. Its first official meeting is in Paris at the end of May. Because it too is part of a global agency, and because it represents international commissions in various disciplines more than national councils, ICSU can acquire an important role outside North America and Western Europe.

It can, for example, involve the East European countries and also the USSR, which is apparently keen to participate in the research under agreed guidelines—the USSR has a committee under Professor Bayev which hopes to produce its own guidelines by the middle of the year. ICSU can also involve important Third World countries, such as India and Nigeria, which cannot be prevented from pursuing the research but which might participate in any programme organised by ICSU on their behalf to help ensure that they take the necessary precautions.

The European Molecular Biology Organisation (EMBO). EMBO is based at Heidelberg, where a P4 facility for international use is under construction.

Britain's GMAG gets going

BRITAIN'S Genetic Manipulation Advisory Group (GMAG) has already given outline approval to more than half a dozen experiments, including one that requires the highest level of containment. For the present, however, the only place where such an experiment can be carried out is Porton, a Ministry of Defence establishment whose future is under scrutiny.

GMAG meets monthly to consider proposals for genetic manipulation experiments which, while awaiting a legal definition, are taken to refer to those in which restriction enzyme fragments of nucleic acids are recombined with the genomes of other organisms in which they are capable of propagation. GMAG's chairman is Sir Gordon Wolstenholme; it has 19 members, of whom only three have a direct interest in recombinant DNA research. Most of the others are biological scientists, but there are four union representatives and two non-scientific academics.

It is GMAG's task to consider any proposals and to state its objections, if any. Proposals (25 copies) are submitted on a formidable form on which full details of the protocol, personnel and facilities have to be provided along with information regarding local safety committees, biological safety officers, medical officers and health monitoring schemes. Proposed experiments fall into four categories, depending on the associated hazards. It is up to the proposers to categorise their own experiments but up to GMAG to ascertain whether the relevant requirements are satisfied.

In the case of the two categories (III and IV) that represent the greatest hazards, GMAG has to carry out a site inspection of the facilities. This they have already done for the category III facility at the MRC Mammalian Genome Unit in Edinburgh. Professor Peter Walker of that unit expresses himself well satisfied with the visit and its outcome which, pending attention to a few minor problems, should give him the first approved category III facility in Britain. Site visits have also been requested for a proposed category III laboratory at Imperial College and for the Microbiological Research Establishment at Porton. It is on Porton that British short-term hopes for a category IV facility hang—though for now on some rather delicate strings. Porton is the property of the Ministry of Defence and has been the laboratory in which much of Britain's microbiological warfare research has been carried out. As such it has long been unloved by many academics. Now it is also unloved by the Ministry, which recently announced that it no longer has a use for the microbiological laboratories. Since December their future has been uncertain and is now the subject of a confidential report prepared by the Central Policy Review Staff for the Cabinet (see Britain, page 9). A decision is thought to be imminent.

There are bound to be problems if the decision leaves the laboratories under the control of the Ministry of Defence. If that happened, it is known that a faction of GMAG, led by some of the trade union representatives, would be strongly opposed to the use of Porton's facilities for civil genetic manipulation. That could prevent the go-ahead of any category IV experiments, including the one that has already been given outline approval, until an alternative category IV laboratory could be found. At the moment none are planned but it would not be too far-fetched to suppose that the proposed category III facility currently being completed at the Beatson Institute, Glasgow, could be upgraded if required.

It is Scottish laboratories that have so far taken the lead in applying to GMAG, with their English counterparts being distinctly slow off the mark. Of the expected initial batch of 40–50 applications less than 15 had reached GMAG by its third meeting last month and at least eight of those were from north of the border, mainly from Professor Walker's laboratory.

GMAG has in fact expressed surprise at the paucity of applications and has reminded those concerned that they would be foolish not to present their proposals to the group. At present application to GMAG is voluntary although the UK Health and Safety Commission (HSC) is redrafting its controversial regulations that would make notification compulsory. The original proposals were strongly criticised for being far too wide-ranging, especially as existing health and safety regulations already cover the laboratory hazards of genetic manipulation in the general sense.

The Research Councils have themselves warned of their intention of withdrawing funding of any rogue manipulators. Only industry would therefore be immune from financial sanctions, but the two most interested pharmaceutical companies have already made their intentions clear by submitting proposals to GMAG.

Peter Newmark

It was the first international European body to become involved in guidelines for recombinant DNA research, and has a Standing Advisory Committee on Recombinant DNA chaired by Professor Charles Weissmann. This was set up in January 1976 and held its first meeting in London the following month to discuss whether it was worth elaborating guidelines, other than those available, for Europe as a whole.

At its second meeting, in London last September, the committee compared the NIH and Williams guidelines. It suggested the establishment of national advisory groups to specify containment measures for each experiment on the basis of a detailed protocol submitted to it. The committee specifically recommended against the idea of using some combination of the procedures from the two sets of guidelines. And it advised that experiments forbidden under the NIH guidelines not be carried out.

The European Science Foundation (ESF). Established in November 1974, the ESF is made up of 45 national research councils and academies from 18 European countries and aims to create a close-knit community of science and research in Europe. Sweden quickly suggested to the founding committee that it should consider the whole question of genetic manipulation, including its social, legal and ethical aspects. With the EMBO committee able only to provide advice on request, and then only about scientific and technical aspects, the ESF decided in October 1975 to broaden a preparatory working party into an ad hoc committee on Recombinant DNA which could propose through ESF members whether and what action should be taken at European level.

The committee met three times in 1976 under the chairmanship of Professor Povl Riis of Denmark; members included molecular biologists, physicians and lawyers. Their brief was broad, and they concluded that the recommendations and code of practice of the Williams report should be adopted as the guidelines for recombinant DNA research in Europe. They also recommended that national registries of research should be established and that laboratories should be legally obliged to declare their work to it; laboratories would adhere to agreed guidelines voluntarily, however, and supervision and monitoring would be a national responsibility. National variations, it suggested, should be minimised.

The ESF has now created a new committee made up of representatives of the geemags of its members with the aim of proposing guidelines for Europe. This European Committee on Recombinant DNA will meet for the first time in Strasbourg on 15 March. It will note differences in the practices of various countries and consider prescribing measures for the future.

The European Commission. The European Commission, which has a dual role in the EEC of both initiating and implementing Community legislation, finally jumped into the fray in January. Spotting the opportunity provided by a potential need for Community-wide legislation and harmonisation, Directorate General XII (Research, Science and Education), headed by Dr Gunter Schuster, called geemag heads to Brussels on 21 January for "informal consultations". This offended some sensibilities, not least because Dr Schuster was seeing members of the new ESF committee on which he is himself the EEC's representative.

The Commission is apparently contemplating a directive, the device by which it can request member states to modify and harmonise their legislation. In the case of recombinant DNA research this might involve asking the Nine to ensure that they take the same precautions, but not interfering with the operations of individual geemags. At the January meeting there was no stern objection to the idea, provided a directive was not too specific or detailed.

The worry is not chiefly about Commission interference, although its record in science is less adequate that it might be. Most people recognise that it possesses the authority both to hasten the necessary harmonisation and to incorporate research done in the private sector a common framework. The worry for the moment is related more to the style and timing of the Commission's involvement, which could be self-defeating if it breeds resentment among researchers.

The outcome of the January meeting was presented last week at a meeting in Brussels of the Medical Research Committee, a sub-committee of CREST, the Commission's Scientific and Technical Research Committee. According to the office of the director of the biology programme, the meeting reached no firm conclusions and is due to meet again only in June. That may mean that EEC invlovement will remain peripheral for a while yet. If so, the immediate burden of recommending a path for recombinant DNA research in Europe now lies with the ESF. □

USA

From Carter via Ford

Colin Norman reports from Washington on President Carter's proposed budget changes

AFTER three weeks of frantically sifting through the massive set of budget proposals which President Ford left behind, President Carter last week sent Congress a raft of major and minor amendments. Designed to implement some of Carter's more prominent election promises and political priorities, the proposed budget revisions would undo some of the Ford Administration's parsimony toward health, welfare and housing programmes and add about $19,400 million to previous estimates of government spending in the 1978 fiscal year (which begins on 1 October, 1977).

As far as science and technology are concerned, the only areas which figured prominently in campaign rhetoric were energy and military research and development, and consequently they are the only areas greatly affected by Carter's proposed budget revisions. Outside those two fields, Carter has proposed a small addition to Ford's budget for NASA for studies of possible follow-up missions to the Mars Viking Lander project, and a $5 million increase in the budget of National Institutes of Health (NIH) for research on childhood diseases. Otherwise, the relatively large budget increases for basic research, earthquake prediction and agricultural research proposed by President Ford have been left untouched.

The proposed revisions to the budget for energy research and development are another matter. Acting on the advice of James Schlesinger, his energy adviser, Carter has scaled down Ford's budget proposals for three long-term energy programmes—the breeder reactor, development of large solar power plants and thermonuclear fusion—and redirected some of the funds towards efforts likely to produce short-term results. The proposals signal a major shift in priorities and indicate that the new Administration is prepared to play a more aggressive role than its predecessor in pushing new technologies, such as solar heaters and electrically powered automobiles, into the market-place. The budget pro-

Press report

It is now virtually certain that President Carter will name Frank Press, a distinguished geophysicist from the Massachusetts Institute of Technology, as his science adviser and head of the White House Office of Science and Technology Policy (OSTP). Though no official announcement had been made by the end of last week, Press was already at work in the White House, helping to select candidates for other top science posts in the federal government and attending meetings of Carter's senior advisers. Administration sources indicated that a formal announcement was imminent.

The appointment of Press, which was first rumoured a couple of weeks ago, is something of a surprise. Although he is no stranger to the Washington science policy network, he is not generally regarded as part of the inner circle of the scientific establishment. It was, in fact, widely expected that the job would go to Lewis Branscomb, head of research at International Business Machines (IBM), who coordinated a science policy task force for Carter during the campaign. It has been suggested, however, that Branscomb was ruled out because several people with IBM connections had already been appointed to top posts in the Carter Administration, a fact which has attracted some criticism.

Press was recommended to Carter by a number of prominent scientists, particularly Defense Secretary Harold Brown and Jerome Wiesner, President of MIT, and his appointment has generally been greeted with warm approval in the scientific community. An outstanding scientist, he was elected a member of the National Academy of Sciences at the age of 33. He was Professor of Geophysics at Caltech from 1955 to 1965 and Director of the Seismological Laboratory for much of that time. Since 1965 he has been Chairman of the Department of Earth and Planetary Sciences at MIT.

Among his credentials for the science adviser post is his wide experience in the science and politics of test ban negotiations. He has long been interested in the problem of identifying the seismic waves from underground nuclear explosions and discriminating them from signals from earthquakes. He was a United States delegate to the test ban conference in Geneva in 1960–62, which paved the way for the partial Test Ban Treaty in 1963, and he has been a consultant to the Arms Control and Disarmament Association. In 1968 he was a member of the international working party convened by the Stockholm International Peace Research Institute which reopened the question of monitoring underground weapons tests. Since Carter has frequently promised to see a comprehensive test ban agreement with the Soviet Union, Press's experience in such matters is said to have been a strong point in his favour.

Other relevant Washington experience includes a stint on the President's Science Advisory Committee (PSAC) from 1961 to 1964, and membership of the National Science Foundation, from 1970 to 1976. He is also Chairman of the National Academy of Sciences' Committee on Scholarly Communication with the People's Republic of China, and has travelled extensively in China in recent years.

Even though Press's administrative burden has grown heavier every year, he has continued to make major and distinguished contributions to geophysics. His early work was concerned with seismic wave propagation in the earth's crust and upper mantle, and this eventually resulted in a book *Elastic Waves in Layered Media* in collaboration with M. Ewing and W. Jardetzky. The book had a great influence on the emerging science of seismology. In the 1960s his interests turned to lunar seismology and he was a member of the Apollo team that discovered moonquakes. In the same period he was also encouraging early US research in earthquake prediction as Chairman of the Earthquake Prediction Panel of the Office of Science and Technology. In the late 1960s he started to work on the problem of inversion of earth data by Monte Carlo methods, and more recently his research has included study of the excitation of the Chandler Wobble by earthquakes. In 1974 he published, with R. Siever, a major geology/geophysics text *Earth*.

Colin Norman

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posals, moreover, are only a foretaste of a comprehensive energy policy which Carter has promised to unveil in mid-April.

The budget revisions suggest that the besieged liquid metal fast breeder reactor (LMFBR) programme is in deep trouble. Carter has recommended that nearly $200 million should be taken out of Ford's budget request for the LMFBR effort next year, a reduction which would still leave $656 million in the programme, but which would cut spending to below this year's level. A statement published last week by the Energy Research and Development Administration (ERDA), which is said to have been drafted by Schlesinger, announced that the entire LMFBR programme will be subjected to an intensive review, to "assess the role of LMFBRs in the nation's energy future, the timing and pace of the breeder programmes and the timing of any decision on whether the breeder would be a viable energy option".

The implications are made clear later in the statement:

> The President's energy priorities, as reflected in the revision of ERDA's (fiscal year) 1978 budget request, stress conservation and nearer-term supply technologies. These priorities suggest that past plans for expansion of the LMFBR program may no longer be viable. Furthermore, serious questions have been raised about the LMFBR technology and the structure of the current LMFBR program. The energy potential of this option must be weighed against the safety questions associated with the LMFBR and the dangers of nuclear proliferation from plutonium reprocessing needed by LMFBRs.

In particular, the review will focus on ERDA's plans to build a demonstration fast breeder reactor on the Clinch River in Tennessee, a project which represents the next major step in the LMFBR programme in the United States. Construction of the Clinch River plant was scheduled to begin later this year, but it will now be delayed pending the outcome of the review. Consequently, Carter has suggested that $85 million be cut from Ford's request for that project alone. No date has been set for completing the review, but it is unlikely that a final decision will be ready in time for inclusion in the energy policy which Carter will unveil in April.

As for solar energy research and development, again, long-term efforts are being cut back to allow more funds to be channelled into programmes likely to produce more immediate results. Accordingly, Carter has scaled down Ford's budget proposal for construction of a pilot-scale solar thermal generating plant, and recommended that the savings be used chiefly to underwrite the costs of producing and testing some 1,300 solar heaters.

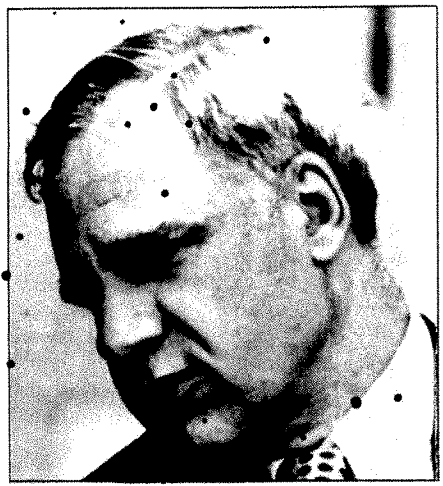
The pilot plant project which Carter wants to cut was only given a go-ahead by the Ford Administration on 7 January. It would entail construction of a 10 MW generating plant in the Mojave Desert in California; the plant would essentially consist of a bank of mirrors concentrating the sun's rays onto a steam generator atop a tower. The project, the costs of which would be shared with electric utilities and government bodies in California, was scheduled for completion in 1980 or 1981. Ford had requested $65 million for the project next year, but Carter has recommended that the budget be reduced to $10 million. The original plan was to follow the California plant with another pilot plant a couple of years later, leading eventually to a commercial-scale plant in the late 1990s. No decision has yet been made on how far the project will be stretched out under Carter's energy policies.

Carter's proposal that more funds be pumped into the production and testing of solar heaters represents a marked change from the policies of the Nixon and Ford Administrations. In 1974, Congress passed a bill to provide federal support for up to 5,000 solar heaters to be installed in public and private buildings and tested over a five-year period. The idea was to stimulate the growth of a solar heating industry, but the Ford Administration was opposed to the principle that the federal government should be so directly involved in the market-place, and the legislation consequently was implemented in a relatively half-hearted manner. Carter clearly shares Congress's view that federal support is needed to commercialise new energy technologies.

Similar views show up in Carter's proposals for energy conservation. He has doubled Ford's budget request for research and development on energy-saving systems, recommending that a total of $318 million be spent next year. Much of the proposed increase would be devoted to programmes designed to increase the efficiency with which energy is used in industry, buildings and transport, but $40 million would also be spent on a programme to provide federal subsidies for the production and testing of electrically powered vehicles. Again, that is an effort which Ford had argued should be the responsibility of private industry rather than of the federal government.

As for the fusion programme, Carter has recommended that $60 million be docked from Ford's request for research on magnetic confinement, leaving $311 million in the programme. He also suggested that Ford's request for laser fusion studies be reduced from $142 to $122 million. Again, the pattern is to decrease support for long-term programmes, but to allow nearer-term efforts to proceed. Thus, the Tokamak Fusion Test Reactor (TFTR), a major facility being built at Princeton University, will be allowed to go ahead, but the completion date would slip by about 6 months, to mid-1981. Similarly, a large magnetic mirror machine planned for the Lawrence Livermore Laboratory would suffer a $10 million cut, but it would also be allowed to proceed with only a minor slip in schedule.

As far as the addition to NASA's budget is concerned, the Carter Administration has unexpectedly recommended that $15 million be spent next year on studies of potential missions to Mars as a follow-up to the successful Viking project; Ford had recommended only $5 million for such efforts. Several candidate missions are under consideration, ranging from refurbishing the back-up Viking spacecraft, which was held in reserve in case the original Viking mission failed, and launching it in 1984, to construction of a new spacecraft to return samples of Martian soil to Earth. The more ambitious missions would probably not be launched until 1988. In any case, planetary scientists are encouraged by Carter's modest budget addition, seeing it as an expression of Presidential interest in the space programme.



Keystone

Energy man: James Schlesinger

The only other budgetary change of note is the fact that Carter has added only $5 million to the budget of the National Institutes of Health. Ford's budget request for NIH was relatively parsimonious, providing an increase of only $40 million, which is insufficient to keep pace with inflation. Congress traditionally adds substantial increases for NIH, however, and the Carter Administration will probably not object if Congress carries on the tradition this year. □

SEVESO

Dioxin damage

Alastair Hay reports on recent developments in Séveso and a possible link connecting dioxin with birth defects.

Official confirmation has now come that at least two of the women who were exposed to tetrachlorodibenzo-*p*-dioxin (dioxin) at Séveso last year have given birth to children with malformations. But the director of health for the Lombardy region, Dr Vittoria Rivolta, is quoted as saying that it is "not yet possible to link the deformations with dioxin contamination". The dioxin was present in the discharge from a trichlorophenol reactor which overheated at the ICMESA chemical plant on July 10.

The first abnormality to be observed was a slight intestinal obstruction in an infant born on 10 January. The mother had apparently eaten garden produce which was heavily contaminated with dioxin. The second case—a "small genital deformation"—was reported on 7 February. But the mother was resident in an area not officially considered to be contaminated by the chemical.

According to Dr Rivolta both infants underwent successful surgery shortly after birth. He added that, in view of the known teratogenic properties of dioxin, the Lombardy regional health department was keeping "a record of all children who are born with deformations from mothers who were in the Séveso area at the time of the explosion".

The developing human embryo is particularly susceptible to teratogenic agents during the period of organogenesis when tissue and organ differentiation occur. In Man this period generally occurs between gestation days 18–55. The children born on 10 January and 7 February would therefore have been at a stage of foetal development beyond organogenesis. For that reason they might be considered to have been less susceptible to the teratogenic properties of dioxin. However, as one mammalian development scientist put it, "We don't know the teratogenic mechanism of dioxin and we have no idea at which stage of its development a foetus ceases to be at risk to this chemical".

School children in Séveso and its

neighbourhood are among the latest victims of chloracne, the skin complaint which can appear as a result of contact with dioxin. The number of children affected is reported to be about 340. Most of them, it seems, have come into contact with dioxin through contamination of school buildings. Indeed, dioxin contamination of the buildings is considered to be too serious to allow the schools to remain open. Following protests from school teachers and parents the Lombardy authorities have closed six schools in the Séveso municipality; they will remain closed until the work of dioxin decontamination is completed.

The decontamination programme itself has come in for much criticism in Italy. Local residents are reported to be incensed over the delay in cleaning the Séveso area and the Italian Parliament is concerned enough to consider appointing a commission to investigate the cause of the delay.

The Lombardy administration's decontamination programme was, in fact, finally approved by parliament only three weeks ago. The proposed plan of action includes the incineration of vegetation, dead animals and polluted topsoil, which is to be removed to a depth of 25–35 cm. This operation should be completed by August, when all the topsoil will have been placed under canvas on a concrete base at a location near the ICMESA plant. The total material to be disposed of is estimated to amount to about 70,000 tons. It will take three years to burn, in a high temperature incinerator which will itself take a year to construct. The whole decontamination programme will, it is estimated, cost £13.5–15 million and will eventually be paid for by Givaudan, the Swiss company which owns the ICMESA plant.

The variability of dioxin contamination has proved a major obstacle for the local authority in formulating its decontamination programme. With some areas considerably more contaminated than others, a detailed relief map of the area has been necessary and this has taken time to produce.

Many residents of Séveso are angry that it has taken so long to devise a graded decontamination programme, with the result that the Lombardy regional government now stands accused of gross mismanagement in its handling of the whole affair. But the outbreak of chloracne among Séveso schoolchildren has resulted in the far more serious charge being levelled that the local authority has minimised the dangers of contamination.

These accusations will now be subjects for consideration by the Parliamentary Commission. For the pregnant women of Séveso, however, no inquiry will relieve their anxiety. If a link is established between the recent infants born deformed and dioxin, the hundred-odd women who were in the first trimester of pregnancy on 10 July and who will reach full term during the next two months face a bleak future. ☐

POLAND

Problem solver

Vera Rich reports on science policy in Poland following the visit to Britain of the Polish Science Minister.

THE recent visit to Britain of the Polish Minister of Science, Higher Education and Technology, Professor Sylwester Kaliski, was essentially a matter of diplomatic routine. No major changes were expected in the existing programme of Polish–British scientific and technical cooperation and educational exchanges, and the immediate practical outcome amounted to little more than the agreement by both parties that such cooperation is of mutual benefit, should be continued and, where possible, expanded. Nevertheless, the Ministerial visit served to highlight and provide an opportunity for discussing several interesting aspects of current Polish policy.

Professor Kaliski told *Nature* that, in addition to 65 "key" problems, Polish science policy incorporates seven "government problems" to be tackled at top-priority level: water resources, coal mining technology, electronics and integrated circuits, copper, building engineering, cancer, and proteins. Two others, energy (including atomic power engineering) and computerisation, are in preparation. In all these fields there are either already technological exchange agreements between Britain and Poland or else approaches have been made by the Poles for such agreements (in certain cases, notably integrated circuits and computers, response is limited by the Co-Com embargoes). Most of these problems lie largely in the field of technology; two, however, cancer and proteins, seem to fall within the sphere of research rather than application.

The inclusion of cancer in such a list of problems is notable. Whereas problems like mining technology or computerisation of production seem amenable to state planning with its targets and schedules, there are no hints of a major Polish breakthrough in cancer research that would make any comparable set of targets more than a list of desideration. Indeed, Poland does not seem to have been assigned a special role in the new Comecon oncological project, which hardly suggests a Polish lead in the field. The explanation may be one of national tradition: Mme Sklodowska-Curie, the discoverer of radium, was a Pole, and the very word radium, to Polish ears, is connected with two roots meaning respectively 'joy' and 'good counsel', attributes associated in the popular imagination with the early hopes of radium as a specific weapon against cancer.

In the field of protein resources, current plans envisage an increase in output of one million tonnes per year by 1980. New sources are being sought, including the production of animal feed from petroleum waste and a programme for eaxploiting the krill resources of the Antarctic. A research ship, the *Professor Siedlecki*, of the Gdynia Maritime Fishing Institute, is at present in the Antarctic carrying out a "scientific reconnaisance" of krill. Last year, the *Professor Siedlecki* brought home krill specimens for investigation by sixteen research institutes in Poland for possible use for human consumption or animal feed.

This year's expedition is testing methods of processing krill. The prospect of krill harvesting on a commercial scale has been causing concern among ecologists in many countries, since krill stands at the head of so many food-chains. Questioned about this, Kaliski stated that at present the krill project was only at the investigation stage and "we cannot tell what its future will be". No ecological problems would arise, he said, for at least ten years. Ultimately, however, there would have to be some kind of international agreement governing the harvesting of krill.

In the current Five-Year Plan, said the Minister, government spending on sciences will be increased by some 70% from 115 thousand million to 200 thousand million zloty. This, he suggested, presumably referring to the involvement of scientists in the current unrest in Poland, is "proof that we don't believe there is a crisis in our country". The financial target is not represented by the budgets to date, which for 1976 and 1977 envisage expenditure on science of 16.6 thousand million and 15.9 thousand million. A considerable increase is to be expected in the last three years of the plan, if the 200 thousand million target is to be reached.

The Minister said that of the total expenditure on science some 12–15% is alloted to fundamental research, and the rest to applied research projects, the latter being financed half from the central budget and half from industry by means of research contracts. The implementation of such applied research in production is Poland's biggest problem. This statement is true also of the Soviet Union, but whereas Soviet scientists frequently receive official rebuke for not producing the results that industry needs, the problem in Poland, says Kaliski, is that they "often have very good scientific results but no chance of introducing them". Poland's scientists, he explained, "think that if they have obtained a result, that it must be introduced". This was not always feasible under a socialist system; under capitalism it would always be possible to find some manufacturer who would undertake to introduce a new process simply for profit motives, but in a system of state planning this was not always possible. To test promising innovations at the pilot stage, he pointed out, the Polish Academy of Science possesses a small pilot factory. The main activity of the Academy, however, is fundamental research and "quality potential", rather than the development of individual applications.

Concerning Comecon, Kaliski said that Poland participated principally in mining, computers, electronics and laser physics. In atomic power engineering, the Poles were "not the leading group—but we all contribute". In the Interkosmos space programme, Poland was concerned mainly with on-board computers, the application of space research to cartography and meteorology, the study of plasma *in vacuo*, magnetic field research, and signal propagation. She was involved in "serious collaboration" in space medicine. Kaliski appeared quite satisfied with the present scheme of financing Comecon research projects by direct contracts between the countries concerned, and showed no interest in the more sophisticated arrangements suggested recently in the Polish journal of international affairs *Sprawy Miedzynarodowe*.

One interesting feature of Kaliski's visit was his meetings with laser physicists. These were not, strictly speaking, in his capacity as Minister. In addition to his governmental duties, Professor Kaliski is head of the Institute of Plasma Physics and Laser Microsynthesis in Warsaw. In an informal lecture which he gave at the Rutherford Laboratory, Chilton, he described the work of the Institute on microthermonuclear synthesis.

BRITAIN

More of the same

The UK Government has published details of its proposed expenditure plans for the next few years and its Defence Estimates for 1977. Chris Sherwell reports

LAST week's White Paper, *The Government's Expenditure Plans, Volume II* (Cmnd. 6271–II, HMSO, £2.35), which follows publication earlier of a general account of the plans, contains the details of individual expenditure programmes. Twelve months ago the government published its plans for the rest of the decade, and these promised a painful levelling off in the growth of expenditure. Since then the government has announced two rounds of further measures, in July and December; the impact of the latter has yet to be assessed and incorporated into figures for 1979–80 onwards.

Unsurprisingly, the latest plans confirm the trend foreshadowed last year. Because organised research and development in Britain has a large government element, this means that the circumstances under which the country pursues its science effort remain far from happy. Some of the details from the White Paper are contained in Table 1 (see over).

For the Ministry of Defence, which is the government's biggest spender on R&D, the table shows only the trend in the overall budget. Latest plans are available in more detail in the *Statement on the Defence Estimates 1977* (Cmnd. 6375, HMSO, £1.50). Expected expenditure on the defence research programme in 1977–78 amounts to £123 million, two-thirds of it devoted to "evaluation and exploratory development" on equipment and weapons, the rest devoted to maintaining a base of scientific knowledge and advancing technology. Another £23 million-worth is linked with civil research.

Defence research did not escape the public expenditure axe last year, and the White Paper confirms this trend too. Implementation of the rationalisation of R&D establishments is described as "well advanced with the first major moves now taking place". By the early 1980s four "systems establishments"—sea, land, air and underwater—complemented and supported by eight "technology establishments", will have replaced the 25 original R&D establishments.

With the reviews of future levels of R&D at Porton complete, work on chemical warfare at the Chemical Defence Establishment (CDE) "can be reduced to about two-thirds its present size", the White Paper says; and the future of the Microbiological Research Establishment (MRE), because microbiological research requirements can be met "by a small team of ten scientists" integrated within the CDE, depends "on the scale and range of civil requirements".

Other details from the White Paper on expenditure include:

- Support for the National Physical Laboratory, the National Engineering Laboratory and Warren Spring Laboratory (the Department of Industry's Industrial Research Establishments), together with department-sponsored work at the Atomic Energy Authority (UKAEA) and the Natural Environment Research Council (NERC), is expected to remain at broadly the same level up to 1980–81. The general industrial research and development programme overall, however, has been reduced by £2 million compared to last year "as a contribution to savings in public expenditure".
- The pattern of funding for R&D in certain aviation fields will be modified if and when the new nationalised industry, British Aerospace, comes into existence with the passage of the Aircraft and Shipbuilding Industries Bill through parliament.
- Reflecting developments in the past year involving the country's nuclear reactor programme, the White Paper says that the provision for R&D by the UKAEA is now "subject to adjustment in the light of the current review" of the programme.
- The Science Budget, which indicates the organised government effort on the civil side in basic sciences, reflects the reductions of last year. Resources devoted by the Science Research Council to 'big science', the White Paper says, will be reduced by about 25% over the survey period. The breakdown for 1976–77 is shown in Table 2.
- In most nationalised industries, but especially electricity and telecommunications, the lower forecasts of investment in fixed assets are the product of revised estimates of demand. ☐

Table 1 Trends in science-related expenditure of UK government (£ million at 1976 survey prices)

	1973-74	1974-75	1975-76	1976-77	1977-78	1978-79	1979-80[1]	1980-81[1]
Defence budget[2]	5,460	5,265	5,548	5,621	5,444	5,397	5,652	5,630
Industrial innovations, of which:	370	359	373	281	224	214	207	301
General industrial R & D[3]	38	39	42	51	56	61	66	68
Technological and industrial sponsorship	6	5	7	8	11	12	12	12
Aircraft and aero-engine R & D	26	25	19	19	13	12	12	12
Concorde development and production	116	102	81	37	34	28	19	12
RB211 and other aircraft and engine	64	76	92	11	−11	−5	−6	−6
Space	18	22	25	30	25	20	20	20
Nuclear	102	90	107	127	97	86	83	83
Research councils[4]	229	232	235	217	216	215	217	215
Expenditure on fixed assets by:								
National Coal Board	122	200	241	293	332	373	360	377
Electricity Council and Boards	657	667	699	704	646	622	586	570
South of Scotland Electricity Board	109	88	73	59	45	66	97	132
British Gas Corporation	182	267	368	259	233	307	345	345
British National Oil Corporation	—	—	53	148	173	210	255	330
Telecommunications (Post Office)	1,004	943	916	917	865	906	881	901
Health and Safety at Work[5]	15	13	26	30	35	33	34	34

[1] Figures shown are those of last year's survey.

[2] About 12% of last year's defence budget went to R & D.

[3] Includes the Department of Industry's Industrial Research Establishments and department sponsored work at the UKAEA and NERC.

[4] See Table 2 for pattern of each Council's Expenditure in 1976-77.

[5] New category, covering allocation for activities of Health and Safety Commission and Executive under the 1974 Health and Safety at Work Act.

Source: *The Government's Expenditure Plans, Volume II* (Cmnd. 6721-II, HMSO, £2.35).

Table 2 Pattern of Council spending for 1976-77 Science Budget (£ millions)

	Agricultural Research Council	Medical Research Council	Natural Environment Research Council	Science Research Council	Social Science Research Council	Total
Research grants and contracts	1.5	11.0	2.2	26.6	4.1	45.4
Research units and establishments	6.8	20.6	17.0	40.8	0.7	85.9
Research Council grant-aided institutes	9.1	0.0	2.0	0.0	0.0	11.1
Postgraduate awards	0.1	2.5	1.9	11.8	5.0	21.3
International subscriptions	0.0	1.1	0.0	31.5	0.0	32.6
Centrally supported schemes and administration	0.8	2.2	2.9	6.5	1.4	13.8
Total	18.3	37.4	26.0	117.2	11.2	210.1

Table does not include expenditure by the Natural History Museum and the Royal Society (£8 million).
Source: *The Government's Expenditure Plans, Volume II*

BANGLADESH

● India's construction of a barrage across the Ganges river at Farakka, about 18 km upstream from the Bangladesh border, is diverting water from the Ganges into the Bhagirathy-Hooghly river. The aim is to flush the silt off the river bed and so keep the Calcutta Port navigable throughout the year. But the project poses a threat for Bangladesh, a densely populated state on a delta whose main artery is the Ganges.

It is estimated that the water from the Ganges and its tributaries serves about 37% of the area of Bangladesh in which about 25 million or nearly one-third of the total population live. About 40,000 cusecs of the Ganges water are redirected into the Hooghly river in dry months, and the effects of such diversion could be severe. Irrigation of 400,000 acres of land dependent on Ganges water was noticeably affected in the last dry season.

According to recent statistics published by Bangladesh Water Development Board, the average discharge of the Ganges at Hardinge Bridge during the last 10 days of December 1976 stood at 74,000 cusecs, as against 47,200 cusecs during the corresponding period of 1975. Before construction of the barrage the flow in this period varied from 118,000 cusecs to 167,000 cusecs; the average flow during March is normally 74,000 cusecs.

Particularly worrying are the rise in the salinity of the water and the extent of saline intrusion into the land from sea. The index of salinity in the Bhairab river went up to 13,600 micromhos per cm^2 in April last year, compared to the historical average of 500 micromhos. The saline penetration inland advanced from the normal 270 km to about 440 km from coast, affecting an area of 6,820 km^2 beyond the limits of past years. The salinity of the Ganges water has since recorded a further rise. In some parts, particularly in Bhairab river, the salinity index has exceeded 15,000 micromhos per cm^2; it may rise further next spring.

The reduced water flow and increased salinity will in addition have deleterious effects on the forest resources of Bangladesh: Sunderbans, the largest forest reserve near the coastal belt, faces the possibility of thinning out and an ecological imbalance for men and animals there. The ecology of aquatic life is also adversely affected: the depletion of water levels in dry months and enhanced salinity have affected the fish population at the key landing sites, and there could be long term effects on spawning cycles, fish size and variety.

● Bangladesh has been elected a member to the 58-member Governing Council of the United Nations Environment Programme (UNEP). The council is one of the semi-autonomous special bodies of the United Nations with headquarters at Nairobi. Asian states have 13 seats in the body; other countries from the Asian Group elected together with Bangladesh are China, Philippines, Indonesia and Syria.

M. Kabir

IN BRIEF

Spacemen return

The latest crew of the Soviet Salyut 5 space station have returned safely after an 18-day flight. The programme of the mission was the now-familiar mixture of astrophysical and geophysical observations "in the interests of the national economy", the testing of on-board instruments and systems, and medical observations. The results of one of the medical experiments, which investigated the threshold sensitivity of the vestibular apparatus to electrical stimuli under conditions of weightlessness, will be used to compile criteria for the selection of future cosmonauts.

Japan has meanwhile launched her first geostationary satellite, on 23 February from the Tanegashima Island Space Centre. Although it is Japan's tenth satellite, it is the first to remain in a geostationary orbit and should bring advances in communications and resources surveying.

Change in Grenoble

Dr John White took over as director of the Institut Laüe-Langevin in Grenoble this week following the departure of Professor Rudolf Mossbauer for a teaching chair at the University of Munich. White's place as assistant director at the Anglo-French-German high flux reactor is taken by Tasso Springer, from the Centre for Nuclear Studies in Julich, West Germany.

EEC geothermal programme

The European Commission is asking for proposals for research and development for the second stage of its geothermal energy programme. The programme, first announced in August 1975, embraces the acquisition of new geothermal data, prospecting methods, and energy from hot water sources, steam sources and hot dry rocks.

Five UK proposals were accepted for the first stage of the programme, including one from the CEGB and two from the Natural Environment Research Council. The EEC will provide about half the cost of the projects involved.

UK reactor choice

Sir Alan Cottrell, the UK government's former chief scientific adviser whose questions about the reliability of the steel pressure vessel of the pressurised light water reactor (PWR) prompted a massive study, has again made public his views about the choice of reactor question. In a letter to the *Financial Times* last week he wrote that Britain "would have little to gain and a lot to lose" by choosing the PWR; he also remained doubtful whether it was right to have to rely for safety on human standards in construction, supervision and operation. He described the merits of SGHWR/Candu and AGR systems as "fairly evenly balanced".

Man management

KENNETH MELLANBY

THE most successful and long-running serial put out on the radio by the British Broadcasting Corporation is *The Archers*, a programme dealing with a farming community in the imaginary village of Ambridge in the midlands of England. Topics of current interest are often introduced: thus Phil Archer, a vigorous and progressive farmer, decides to go on a management course organised by the official Agricultural Training Board. His wife is only being mildly ironical when she says that this will teach him to "avoid friction and frustration with the men, teach a logical approach to human problems, improve their job performance, create team spirit and good morale, and develop effective face to face communication". The even more successful farmer to whom this was said replies: "It won't leave much time for farming". This episode may appeal to the many scientists who are being pressurised to attend similar courses, organised by the training branches of government departments and research councils. They don't leave much time for research.

Most people would agree that many branches of science are developing so rapidly that it would be easy to get out of date, and that some kinds of 'refresher courses' may be useful. However, good scientists usually manage to keep abreast of their subject, and even find little difficulty in dealing with the massive literature reporting new work. It has always been the practice in the better institutions for staff to be encouraged to visit other laboratories where advances are being made, and to attend special courses to learn new methods and techniques.

Instruction in such scientific advances is seldom the concern of the training branches which are now becoming so widespread. They are mainly involved in promoting the teaching of 'management'. In this, they have been encouraged by reforms in the scientific civil service following the Fulton Report; changes intended to improve efficiency and promotion prospects. Trades unions have also pressed for this type of course, believing that it may enhance their members' prospects, even if there are doubts as to whether those attending obtain any real benefit.

I have had the opportunity of trying to assess the effects of these management courses on a good many of my colleagues—men and women employed primarily to do research. I have been unable to detect any improvement in their originality or their productivity, or even in their ability to handle their juniors (or their seniors). The courses seem totally useless in promoting scientific research, they waste time which should be spent in the laboratory and they use scarce funds needed for the research itself. I have found this view shared by the directors of many other establishments.

However, the hope is often expressed that, at a suitable stage in their careers (perhaps when their originality is flagging) more scientists will move to senior administrative posts, and that those who have studied management will then be at an advantage. Has anyone ever carried out a controlled experiment, comparing the performance of the 'trained' and the 'untrained'? I have seen no improvement in those who remain in research. Do these management courses really promote effectiveness in scientific administration—or, for that matter, in business or industry? Until these questions can be answered, we should surely be cautious in encouraging these developments. We should certainly not spend money intended for research, except perhaps to do the research needed to evaluate the courses themselves.

news and views

Some singular proposals

from P. C. W. Davies

ONE of the more intriguing aspects of the theory of relativity is the prediction that the theory itself will inevitably break down in certain plausible situations. When this happens, spacetime is said to develop a singularity. Usually, singularities are associated with intense gravitational fields which grow without limit, such as may occur after the catastrophic implosion of a star to form a black hole. Physicists have always been disturbed about singularities, because if they are taken literally, they imply that there can be regions of the Universe where spacetime develops an edge or boundary, at which all of known physics breaks down. Many have recoiled at this possibility, and view the singularity, less catastrophically, merely as an indication that classical relativity ceases to apply under some very extreme conditions. In the alternative tradition singularities are regarded as actual physical entities of some sort, presumably with their own laws and properties. The issue is of literally cosmic importance because it is widely supposed that a singularity lay at the initiation of the big bang primaeval phase of the Universe. If this singularity is real, it represents a past temporal extremity of the Universe, which, in somewhat anachronistic terminology, means that there was once a creation. If, on the other hand, the singularity is really just a jargon word for a new region of the physical world about which we are presently ignorant, then the Universe will be infinitely old, with all that implies for thermodynamic paradoxes and time asymmetry.

Often the distinction between these alternative viewpoints is blurred. However, Stephen Hawking of Cambridge University, who is never one to be faint-hearted in facing tough questions squarely, has made some challenging proposals about the nature of singularities, particularly with regard to black holes. In a thought-provoking paper entitled 'Breakdown of predictability in gravitational collapse', (*Phys. Rev. D.* **14**, 2460; 1977) Hawking argues for the inevitability of singularities and proposes to make a virtue of them by introducing a new physical principle to stand alongside the Heisenberg uncertainty principle. He claims that the breakdown of physics at singularities does not merely signal our ignorance of the correct theory but represents "a fundamental limitation to our ability to predict the future" a limitation which is in addition to the usual quantum uncertainty. This he calls the principle of ignorance.

Hawking discusses in detail a number of situations in which the principle of ignorance can be applied. In all cases there is an intimate connection between singularities and so-called 'hidden surfaces'. General relativity permits the causal structure of spacetime to develop features such as event horizons, beyond which no information is available. In the case of the gravitational collapse of a star the formation of an event horizon leads to the famous thermal evaporation process discovered by Hawking in 1974, and which endows the black hole with a temperature and entropy. The black hole entropy can best be understood as the loss of information behind this horizon. The main part of the paper is concerned with a detailed mathematical treatment of the evaporation process, and confirms the independent work of Parker and Wald that this radiation is truly thermal in the sense that the event horizon appears to emit with equal probability all configurations of particles consistent with the observers' limited knowledge about the black hole (its mass, charge and angular momentum). The precise connection between the horizon and the singularity is a subtle one, and Hawking's argument seems to depend upon an interpretation of the evaporation process in terms of Feynman's idea of antiparticles being particles travelling backwards in time. The evaporation process entails the creation of particle–antiparticle pairs outside the horizon with one particle falling into the black hole (and thence into the singularity) and the other perhaps escaping to infinity to contribute to the evaporation radiation. Hawking regards this situation as a single particle emanating from the singularity backwards in time, tunnelling to the region outside the black hole, where it scatters off the gravitational field into the future direction, and thereby arrives at infinity. In this way the accepted randomness of the evaporation radiation can be regarded as reflecting a property of the singularity, and is hence a direct consequence of the principle of ignorance. One feature which makes me uneasy about this picture is its dependence on an intuitive, semi-classical idea of particles close to, and inside the black hole, even though the quantum wavelength of the particles is comparable to the size of the black hole itself.

Another situation considered by Hawking, which does not involve an event horizon, concerns the big bang creation of the Universe. Here the hidden surface means any surface sufficiently close to the initial singularity about which we have no previous knowledge. The particles emerging from the singularity will then, according to the principle of ignorance, be random and uncorrelated. This idea is not of course new and has frequently been invoked to account for the observed time asymmetry of the Universe (such as the absence of advanced radiation coming from the big bang). Hawking argues that the characteristics of the cosmic thermal microwave background be explained this way. For example, the high isotropy is beautifully accounted for by noting that an anisotropy can be regarded as the presence of a very large excess of long wavelength gravitons in some modes, which, according to the principle of ignorance, is overwhelmingly improbable.

At a technical level, Hawking points out that the loss of information behind the event horizon of a black hole prevents us from using a pure state to describe the quantum system. The conventional procedure of introducing an S matrix therefore fails when black holes are present. Instead one has to deal with density matrices. This modification could be profound in quite general circumstances if quantum gravity effects are taken into account, because virtual black holes might form out of high energy virtual photons when the wavelength approaches the Planck length.

With such a concentration of provocative new ideas, this paper is bound to generate a great deal of debate about fundamental physics. In particular, Hawking's argument for the inevitability of singularities will be challenged by those who have long sought to avoid them. They could be avoided if quantum matter effects can lead to the violation of the so-called energy conditions. Indeed, negative energies and pressures have long been known to be possible in quantum field theory.

The conclusion which Hawking draws is stated with characteristic wit and daring. There is a new level of uncertainty or randomness in physics because "God not only plays dice, He sometimes throws the dice where they cannot be seen". ☐

Genetic modification of plant cells: a reappraisal

from Edward C. Cocking

CELL genetics is now one of the most rapidly developing areas of plant biology, stimulated by the considerable improvement over the past decade in our knowledge of plant tissue and cell culture, and in particular the availability of isolated plant protoplasts. Genetic modification as a result of the fusion of isolated protoplasts and the regeneration of somatic hybrid plants has advanced dramatically in the past five years.

One of the more controversial aspects of recent developments in genetic modification has been the question of the modification of plant cells by exogenous DNA (see for example Ledoux *Nature* **249,** 17; 1974). The general view now is that the present genetic evidence for the modification of plant cells by prokaryotic DNA is circumstantial and weak. Kleinhofs *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **72,** 27481; 1975) concluded that contamination of plant material by bacteria was the likely explanation for what had been interpreted as evidence for the integration of exogenous DNA, as related to the appearance of "hybrid" bands on centrifugation, since no "hybrid" bands ever turned up provided a stringent sterilisation procedure was used. A re-examination last year by Redei *et al.* (*Proc. Int. Course on Cell Genetics in Higher Plants* (eds Dudits, D. *et al.*) Académiai Kiadó Budapest, 1976) of the correction of genetically thiamineless *Arabidopsis* mutants by bacterial DNA is of particular interest. Using supposed DNA-corrected mutants, supplied by L. Ledoux and others, these workers concluded that the 'corrected mutants' were mechanical contaminants either of Langridge's mutant 1018–6, or other mutants, or were wild type. No evidence of DNA-mediated correction in the material examined could be found. So it must now be recognised that even though the work from Ledoux's laboratory has been a great stimulus, on present evidence there are explanations other than genetic modification for the results obtained.

Somewhat similar conclusions can be reached regarding the work of Hess and collaborators. They observed that treatment of a white-flowering pure line of petunia with the DNA of a red-flowering anthocyanin-synthesising pure line results in a genetically stable correction for anthocyanin synthesis in 0.06% of the treated plants. Bianchi and Walet-Foederer (*Acta botan. neerl.* **24,** 1; 1974) have cogently argued that Hess

Serum thymic factor

from A. J. S. Davies

THE function of the thymus as one of the controlling organs in the ontogeny of immunological responsiveness was defined in the early 1960s. The argument about the manner in which this function is exercised has sputtered ever since. The protagonists of the view that cell outflow from the thymus is largely responsible engendered the concept of the 'T' lymphocyte of thymic origin which is part of our contemporary mythology. The alternative notion, that the thymus functions as an endocrine organ, has led a more chequered life. Its latest form is that the thymus does exert a humoral influence but on lymphocyte populations inside the thymus and perhaps on T cells outside the organ. As J.-F. Bach *et al.* indicate in this issue of *Nature* (page 55) the idea is attractive but the evidence has so far not been compelling.

Bach and his colleagues have concentrated on the isolation and characterisation of a thymus-dependent entity in the serum of pigs. Their material, which they designate *facteur thymique serique* (FTS), they find to be a nonapeptide of determined sequence which they have now synthesised.

The test system used during the isolation procedure of Bach and his colleagues relied upon the capacity of FTS to influence spleen cells from thymusless mice in the sensitivity of their rosette-forming capacity to inactivation by either azathioprine or anti-theta antiserum. This assay is, as Bach himself says, esoteric and it may or may not reflect the manner in which the same material influences cells *in vitro*. In other studies it has been found that partially purified FTS can induce theta positivity, that it can alter the cytotoxic potential of T cells from thymusless mice, that it can sometimes enhance mitogenic responses of lymphocytes and that it can influence the immunological reactivity of the autoimmune NZB mice. All this evidence suggests that FTS can affect T cells, perhaps by modification of their cell membrane, but is far from the proof of either the existence or identity of a thymus hormone and Bach correctly refrains from making such a claim.

Bach's approach has been unusual in concentrating on serum as a source of 'thymus hormone'. It will now be possible to determine whether materials similar to FTS can be obtained by squeezing the thymus itself. Also injection of labelled synthetic material will allow its tissue predilections, if any, to emerge. It would be ironic, but not without precedent in the endocrine system, if FTS production was not in the thymus, but only regulated by it.

FTS will doubtless be widely synthesised and may, like transfer factor, be used for a variety of clinical purposes. There are very many factors which either emanate from or are dependent upon the various components of the lymphoid system. Their disentanglement will rest upon the extent to which their characterisation can be achieved. Bach, in presenting a sequence for a small readily-synthesised biologically active peptide, will certainly engender much fluttering in the lymphocyte dovecotes. The effects of this disturbance could be considerable. ☐

did not take the existing differentiation of the shoot apex into account, when he attempted to interpret his experimental results, and that other explanations are possible for the effects observed (for a detailed critical discussion see Hess in *Plant Cell, Tissue and Organ Culture* 506 (eds Reinert, K. J. & Bajaj, Y. P. S.) Springer-Verlag, Berlin-Heidelberg-New York, 1977).

The recent report by Zryd (*Abstract 191, 9th Int. Conference on Plant Growth Substances, 1976* (ed. Pilet, P. E.) that sycamore cell suspensions plated at low cell density at certain stages of the growth cycle are able to utilise lactose as a sole carbon source is now stimulating a reappraisal of other work using transducing phage to introduce selected bacterial genes into plant cells. Using λp*lac5*-treated sycamore cell suspensions Johnson *et al.* (*Nature New biol.* **244,** 105; 1973) concluded that the *lac* genes were expressed, leading to the formation of enzymes which confer the capacity to assimilate lactose. They stated that their untreated sycamore suspension cultures were unable to use lactose as a sole carbon source.

Somewhat similar experiments using tomato callus have been carried out by Doy *et al.* (*Biochemistry of Gene Expression in Higher Organisms* (eds Pollak, J. I. & Wilson Lee, J.) Reidel, New York, 1972). These results were particularly persuasive, as various bacteriophage controls were used. Inoculation with λp*gal*$^+$ enabled callus to grow slowly on 2% galactose medium for up to 15 weeks, whereas untreated cells and the controls (λp*gal*$^-$ with an amber nonsense mutation in the *gal* operon) died within 3 weeks. In the light of Zyrd's results these experiments should be reassessed; larger populations of cells need to be screened to determine whether tomato, and sycamore, can grow on galactose, and lactose, as a sole carbon source in certain cultural conditions.

Such alternative explanations for the phenomena observed, which have nothing to do with genetic modification, should be rigorously explored. Although it has been claimed that in tomato callus treated with Φ80p*lac*$^+$, specifically *E. coli* β-galactosidase is synthesised, no such positive indication was obtained by Johnson *et al.* It is claimed that the biochemical–immunological test of specific protection at 60 °C is regarded as definitive at the molecular level for bacterial β-galactosidase, but this statement also needs critical appraisal, particularly since it is known that plant β-galactosidases are present in sycamore cells (Keegstra & Albersheim *Plant Physiol.* **45,** 675; 1970), and probably also in tomato callus.

A more promising avenue of approach is the possible use of bacterial plasmids rather than transducing phage for the transfer of prokaryotic DNA to plants. The limitations of the present work on genetic modification as a result of DNA uptake are now evident, but there does still exist the possibility of achieving the desired goal. It is now clear that crown-gall disease caused by *Agrobacterium tumefaciens* is the first example of a natural system in which genetic elements of bacterial origin (the Ti plasmids) play a part in the regulation of gene expression in eukaryotic cells (Schell *et al.* in *Molecular Biology of Plants* (ed. Rubenenstein, I.) Academic, New York, 1977). This raises the possibility of using the Ti plasmids for introducing specific bacterial genes into plants.

The most interesting applications would be the integration of the nitrogen-fixing genes (from *Rhizobium* and other bacteria) into crop plants. The *nif* genes of *Klebsiella* have recently been translocated onto the RP4 factor (Dixon *et al. Nature* **260,** 268; 1976). Schell and his colleagues have suggested that this RP4-*nif* factor could be integrated into the Ti plasmid, which could then be assessed for its ability to make plant cells nitrogen fixing. □

Sexual transmission of hepatitis B

from Arie J. Zuckerman

SENSITIVE techniques for detecting hepatitis B markers have over the past few years revolutionised epidemiological concepts of this infection. The estimated 120 million carriers worldwide cannot be accounted for simply by transmission through infected blood and blood products and the question of whether an apparently healthy carrier can frequently transmit the infectious agent by any other means is now of crucial importance.

Hepatitis B surface antigen, a virus marker, has been found repeatedly in human saliva, menstrual and vaginal discharges, seminal fluid and milk. It has also been reported but not confirmed in bile, sweat and tears. So it is not surprising that contact-associated hepatitis B is apparently of major epidemiological significance, and various aspects of the growing evidence for intimate transmission of the virus were discussed at a postgraduate symposium on hepatitis B held as part of the American Association for Study of Liver Disease meeting last November.

Studies on transmission by intimate contact have however produced varied and sometimes conflicting results. In particular studies on possible transmission by sexual contact have produced ambiguous results. Since the surface antigen has been detected in menstrual blood, vaginal secretions and semen, it is possible that the virus could cross mucosal surfaces during intercourse.

The first indications of possible sexual transmission came some 50 years ago when the husbands and wives of known hepatitis B carriers developed acute hepatitis. Recent studies on the sexual transmission of hepatitis have yielded a wealth of good circumstantial evidence, but also conflicting and sometimes ambiguous information. For example, Mosley (*Am. J. med. sci.* **270,** 253; 1975) reported that as many as 18% of the spouses of patients with acute hepatitis B, but none of their other close family, contracted the infection. Heathcote and Sherlock (*Lancet,* **i,** 1468; 1973) also concluded that sexual contact or close domestic contact seemed to be the most likely source of hepatitis B infection in 40% of 67 patients studied. But a similar frequency of hepatitis B surface antigen was found in registered prostitutes in Athens and in control pregnant women of similar age and socioeconomic status; in prostitutes in Germany and mentally retarded patients in institutions, and among prostitutes and nuns in Columbia.

Nevertheless, several studies carried out in London by a team from the Middlesex Hospital seem to substantiate the possibility of sexual transmission of hepatitis B infection. A high prevalence of surface antigen and surface antibody (the latter providing evidence of infection with hepatitis B virus) has been found in patients attending venereal disease clinics. Promiscuity and in particular homosexual promiscuity emerged as factors of special importance. Thus in one of the studies, homosexuals were found to carry the surface antibody seven times more frequently than their male heterosexual counterparts. In another survey antigen was found ten times more frequently in patients attending a venereal disease clinic in London than in volunteer blood donors (bearing in mind, of course, that these groups are not strictly comparable). Again it was noted that the highest incidence of hepatitis B surface antigen was among male homosexuals.

In the most detailed study to date, of over two thousand persons in New York, Szmuness *et al.* (*Annls intern. Med.* **83,** 489; 1975) evaluated two separate aspects of the role of sexual behaviour in the spread of hepatitis B: first, the possibility that this infection can be transmitted by vaginal intercourse and therefore that hepatitis B may be regarded as a venereal disease;

and second, the indirect impact of certain sexual practices on the spread of hepatitis B virus in various population groups. Serological evidence of infection was sought by very sensitive laboratory techniques. A significant excess of hepatitis B infection was found in two promiscuous groups: patients with venereal diseases and their unrelated sexual contacts (15–18%); and male, but not female, homosexuals (37–51%) (volunteers contacted through homosexual organisations). The prevalence of antigen among male homosexuals was 13 times higher than in the controls, and surface antibody was found 4–5 times more frequently than in the controls, 26 to 28 per cent of spouses of asymptomatic antigen carriers were infected; an infection rate 2–3 times greater than that among spouses of the controls.

Serological evidence of infection with hepatitis B virus was found more frequently in individuals who had a higher than average probability of exposure to potentially infective partners or whose sexual behaviour made such exposure more likely. Large numbers of sexual partners, long duration of homosexuality and predominantly rectal intercourse all contributed to a higher frequency of infection. No antigen carriers were detected in the admittedly small lesbian sample, although antibody was found at the same frequency as in heterosexual women in the general population. This low prevalence may be related to the comparative numbers of sexual partners; in the homosexual sample surveyed the mean number of partners during the preceding six months was 1.8 for women compared with 20 for men.

An unexpected finding reported by Szmuness and associates (*op. cit.*) was the high prevalence of hepatitis B in homosexuals whose sexual behaviour included rectal intercourse, and a relatively low prevalence in homosexuals with practices beyond the bounds of the anogenital system. The explanation for this difference may lie in the reported high incidence of recurrent genital and perianal skin and mucosal sores in the former group.

However, it is extraordinarily difficult to prove by such a cross-sectional retrospective study that hepatitis B can be transmitted venereally in addition to the many other potential modes of transmission. Not only is it difficult to collect reliable information on sexual behaviour, but spouses and highly promiscuous groups differ from each other in many respects, posing problems which cannot be overcome by statistical methods. Nevertheless, several studies have now confirmed that in certain groups intimate contact with large numbers of sexual partners and extravaginal intercourse may be responsible for a substantial proportion of clinically apparent or inapparent infection with hepatitis B virus. But it still could not be proven that type B hepatitis can be transmitted by vaginal intercourse. H. O. Conn, in a paper delivered at the American Association for Study of Liver Disease meeting, pointed out that "it must be borne in mind that people who sleep together may do other things as well. They may share eating utensils, razors, toothbrushes, skin abrasions, needles, lovers and bed bugs; and any of these or others could permit the intimate, but non-venereal transmission of viral hepatitis". ☐

Super-rotating atmosphere of Venus

from Garry E. Hunt

THE atmosphere of Venus is huge, composed mainly of carbon dioxide with ubiquitous clouds obscuring the surface which is characterised by temperatures of about 730 K and pressures of nearly 100 atmospheres. Centuries of visual observation provided little more than frustration to the early astronomers with the regular sight of a yellow, featureless cloud deck, which extends to nearly 100 km above the planetary surface, in complete contrast to Earth which averages about 50% cloud cover. But planetary observations in ultraviolet light have provided the most exciting and unexpected result which still remains today as a major unsolved problem in Venusian meteorology. The upper atmosphere in the neighbourhood of the cloud tops super-rotates. Features in this region move in a retrograde manner with a rotational period of only 4 d which is incredibly short when compared with the planet's own solid-body retrograde rotation of 243 d. So Venus possesses the largest ratio between the atmospheric and planetary rotation rates of any body in our Solar System.

Initial evidence of this atmospheric rotation came from the apparent motion of dark features seen in ultraviolet photographs, characterised by Y and ψ shapes centred on the equator (Boyer & Camichel *Annls Astrophys.* **24**, 531; 1961). But is it really a mass motion that is observed? A wave phenomenon propogating around the planet with a phase velocity corresponding to a 4-d period could produce a similar effect. Although Young (*Icarus* **24**, 1; 1975) considered the super-rotation an illusion, a travelling wave-like disturbance driven by the ultraviolet albedo differences, spectroscopic measurements of winds by Traub and Carleton (*J. atmos. Sci.* **32**, 1045; 1975) and the results of the Venera 8 Doppler drift experiment (Marov *et al. J. atmos. Sci.* **30**, 1210; 1973) provide strong evidence that the visible parts of the Venusian atmosphere really are moving rapidly with respect to the planetary surface. The measured wind velocity is large enough to account for most, if not all, of the 100 m s^{-1} apparent equatorial motion seen in the ultraviolet markings

But how persistent are the observed features? According to Boyer and Guerin (*Icarus* **11**, 338; 1966) the Y feature persists for decades at essentially the same longitude in a coordinate system rotating with a period of 4.067 d. On the other hand, Beebe (*Icarus* **17**, 602; 1972) and Scott and Reese (*Icarus* **17**, 589; 1972) thought that owing to the selective nature of the ground-based observations, the features may actually appear at random longitudes and dissipate after 8–16 d. The high resolution images taken during the Mariner 10 flyby are valuable observations in the study of the lifetime of these features, since Dollfus (*J. atmos. Sci.* **32**, 1060; 1975) has shown them to be representative of the more extensive time series of Earth-based measurements. Belton *et al.* (*J. atmos. Sci.* **33**, 1363; 1976) found that the large scale markings (~ 1,000 km) situated between ±45° latitude have lifetimes greater than 4 d and more with an apparent angular motion at the equator. Smaller scale markings (100–500 km) were found to have lifetimes between 1.5 and 4 d. These observations suggested to Belton *et al.* that the Y and polar ring features seen in the images are both visible manifestations of propagating waves.

But we are still faced with the fundamental question—what is the mechanism(s) capable of generating these large zonal winds? The overhead motion of the Sun relative to an observer fixed on the surface is prograde with a speed of about 4 m s^{-1}. Any theory must then account for the retrograde flow of the order of 100 m s^{-1}. Gold and Soter (*Icarus* **14**, 16; 1971) proposed that the upper atmosphere circulation is a result of a solar torque on a thermal semidiurnal tide. Winds of the observed magnitude could be produced in this way only if the momentum transport were molecular. If, however, turbulent eddies provide the main source of viscous friction, then the mechanism is incapable by several orders of magnitude of accounting for the observed winds.

Schubert and Whitehead (*Science* **163**, 71; 1969) suggested that the 4-d circulation is driven by the overhead motion of the Sun relative to the planet's surface. This is known as the "moving flame mechanism". The solar energy is absorbed in the visible cloud tops situated in the upper troposphere and its effects conveyed to higher levels by various transport mechanisms. Because these mechanisms do not act simultaneously the horizontal movement of the source of heat produces a tilt in the vertical pattern of convection. Associated with the tilt are the Reynolds Stresses which, by transporting horizontal momentum upwards or downwards produce and maintain an altitude-dependent streaming motion. This viscous action transfers horizontal momentum vertically at an equal rate in the opposite direction.

General circulation models, such as those which have been applied to the Earth's atmosphere suggest a numerical approach to the study of atmospheric dynamics. Their first application to the problems of the upper atmosphere of Venus was not very successful (see for example Kalnay de Rivas *J. atmos. Sci* **30**, 763; 1973) producing maximum winds of only 8–10 m s^{-1}, which is an order of magnitude less than those observed. Young and Pollack (*Science* (in the press, 1977)) now report a more satisfactory study producing the observed retrograde mean zonal wind of 90–100 m s^{-1} corresponding to a 4-d rotational period. At an altitude of 60 km their model results show meridional velocities of 10–20 m s^{-1} at mid and high latitudes, a 12 K average equator–pole temperature difference and 3 K day–night temperature difference with the night side hotter. These characteristics agree reasonably well with the observations. Young and Pollack suggest that the large zonal winds in the Venusian atmosphere are the result of a non-linear instability, although the rotation of the planet and the motion of the Sun relative to the surface (moving flame principle) play important parts in determining the direction of the mean winds generated from an initially stationary state. Thompson (*J. atmos. Sci.* **27**, 1107; 1970) first indicated the importance of this instability mechanism which results from the perturbation to a steady convective pattern caused by the superimposing of a vertical shear on this original flow. In the Venusian atmosphere, the relatively large shear is the result of the vertical distribution of solar energy, which is mainly deposited above the 40 km altitude.

The latitude dependence of the mean flow produced by the Young and Pollack model corresponds to a constant angular velocity at a given vertical level. This disagrees with the Mariner 10 observations, which show a more complicated picture of an increase in angular velocity from the equator to a latitude of approximately 50° (Murray *et al. Science* **183**, 307; 1974), where the rotational period may be as short as 2 d. Spiral streaks, which may be interpreted as 'jet streams' show in the Mariner 10 images the flow towards the polar vortices where the kinetic energy of the motions is eventually dissipated.

The return flow of atmospheric gas may occur at deeper levels in the atmosphere in order to maintain the planetary angular momentum balance. We do not know the vertical extent of these motions, whether the stratospheric rotation is a separate overturning cell or whether it is linked somehow to a deep stirring of the huge Venusian atmosphere. Sidi (*Icarus* **28**, 359; 1976) found that a long and narrow striplike cloud formation located within the equatorial region and parallel to the equator appeared to move differently from other clouds in the same area. This suggests that inhomogeneities in the upper atmosphere may exist at two altitude levels at least.

Why is meteorology of the upper atmosphere of Venus so different from that of Earth? The super-rotation requires an efficient high altitude thermal forcing, but for the Earth, the largest amount of available radiative energy is that absorbed at the surface. Could other planetary atmospheres exhibit this excess rotation? Leovy (*J. atmos. Sci.* **30**, 1218; 1973) suggests that any atmosphere where heating tends to produce an equatorial thermal bulge, but which cannot develop instabilities because of slow rotation or large damping, must develop an excess rotation in the same sense as the planetary rotation at a high level. The super-rotation of the Earth's thermosphere reported by King-Hele *et al.* (*Planet. Space Sci.* **18**, 1433; 1970) may be another example. □

Muscle diseases at Birmingham

from E. J. Thompson

The Seventh Symposium on current research in muscular dystrophy organised by the Muscular Dystrophy Group of Great Britain was held at the University of Birmingham on 5–7 January 1977. It consisted of papers invited from workers supported directly by grants from the Muscular Dystrophy Group, and was organised by Professor J. N. Walton, Chairman of the Muscular Dystrophy Group of Great Britain and Professor Sir Andrew Huxley, Chairman of the Medical Research Committee.

AT the meeting various aspects of the development of innervation and muscle biochemistry as well as metabolic, immunological and genetic aspects of human muscle diseases were discussed.

One of the basic questions addressed was—how does nerve leave its molecular footprint on muscle? During normal development, muscle is highly and uniformly sensitive to acetylcholine over its entire surface. However, after innervation, sensitivity is decreased everywhere except at the site of nerve contact. With denervation, the muscle reverts to its less differentiated state, yet the original site of nerve contact remains more sensitive than the surrounding membrane. C. R. Slater (Newcastle General Hospital) described an elegant model which allows systematic study of this phenomenon (work done in collaboration with T. Lømo (University of Oslo)). This involves the implant of a second nerve which requires only 3 days contact with the muscle before it leaves the characteristic cluster of acetylcholine receptors in higher density than the contiguous membrane. One can then ask what is the nature of the molecular cement which maintains the peculiar quarternary structure of these receptor proteins?

G. Vrbova (University College London) compared the difference in the chick embryo pattern of innervation between fast and slow muscle (that is, single rather than multiple synapses per fibre) respectively. She noted that fast muscle was less sensitive to acetylcholine generally and has synapses with a larger space constant. It is thus possible that during normal development the nerves which are destined to induce fibres to become fast muscles exert their particular influence by depolarising a larger area along each muscle fibre.

The increasing use of tissue culture to investigate myogenic cell behaviour in defined conditions has necessitated single cell assays for specific gene products. One such probe has been directed against messenger RNA. K. W. Jones (Institute of Animal Genetics, Edinburgh) has isolated mRNA from heavy polysomes which synthesise two heavy chain myosin molecules. By making a labelled copy of complementary DNA, one can then show selective binding to fixed myotubes and to the cytoplasm between fusing myoblasts. There was no binding to fibroblasts in the same culture. Another specific probe is antibody directed against the individual polymorphic forms of myofibrillar proteins. S. V. Perry (University of Birmingham) has shown that antibody to α-tropomyosin as well as that against troponin T will detect individual fibres in a mixed muscle. Further work on β-tropomyosin as well as troponin I should be forthcoming.

In the discussions on human muscle disease J. A. Simpson (Southern General Hospital, Glasgow) reviewed the evidence that myasthenia gravis is an autoimmune disease. Although there have been some difficulties in transferring the complete myasthenic effect into animals, J. Newsom Davis (Institute of Neurology, London) and R. Miledi (University College London) have found that neuromuscular transmission can be blocked *in vitro* by sera from myasthenic patients. However, Newsom Davis also stated that the level of antibody against the acetylcholine receptor may not closely parallel the clinical status of the patients.

There are several good reasons for considering the role of the cell surface in these diseases. P. D. Smith (Charing Cross Hospital, London) reported that patients with polymyositis had circulating lymphocytes with an increased affinity for intact cultured myotubes and a provocative single patient with myasthenia gravis had a five-fold increase in preferential attachment to the muscle. W. J. K. Cumming (Newcastle General Hospital) found that histocompatability group BW14 was increased ($P<0.001$) in a group of patients with polymyositis. R. Yasin (Institute of Neurology, London) showed an abnormal morphology and behaviour (clustered cells) in cultured muscle biopsies from Duchenne patients.

There are certain reservations as to how closely the mouse model of dystrophy parallels the human situation, but there are, nevertheless, several useful parameters which can be further pursued with this system. M. R. West (Newcastle General Hospital) found that dystrophic mouse, (*dy* 1) muscle appeared to reach a lower level of differentiation than normal muscle in a number of different culture conditions.

The pendulum is clearly swinging back from the neurogenic to the myogenic hypothesis of muscular dystrophy. Recent findings now show abnormalities in the dystrophic muscle without exogenous nerve. The most exciting implications derive from abnormalities on the cell surface which could be linked to both intrinsic biochemical abnormalities as well as a general defect in cell–cell signalling. The question remains as to why there should be pseudohypertrophy of the muscle, that is, proliferation of abnormal amounts of interstitial cells. □

Vitamin D at Asilomar

from Alastair Hay

The Third Workshop on Vitamin D was held in the Asilomar Conference Grounds California, from 9–13 January 1977. The Proceedings of the Workshop, which was organised by A. W. Norman, will be published in full by Walter de Gruyter Co., New York.

The exact mechanism of action of vitamin D (D_2 and D_3) still remains unresolved nearly 60 years after Mellanby reported the presence of an anti-rachitic factor in cod-liver oil; considerable progress has, however, been made over the past 10 years.

It is well known that ultraviolet irradiation of 7-dehydrocholesterol in the skin will ultimately produce vitamin D_3 (cholecalciferol), but the *in vivo* mechanism of production is not well understood. M. F. Holick (Massachusetts General Hospital, Boston) has now shown that the initial product isolated from the skin of rats given no treatment other than ultraviolet irradiation is previtamin D_3, thus demonstrating for the first time that *in vivo* the pathway is at least 7-dehydrocholesterol → previtamin D_3 → vitamin D_3.

Until a few years ago there was no reliable assay for the measurement of the dihydroxy metabolites of vitamin D_3 at the physiological level. Now, however, there are two alternatives for the active hormonal form of vitamin D_3, 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), the assays of Brumbaugh *et al.* (*Science* **183,** 1089; 1973) and Eisman *et al.* (*Science* **193,** 1021; 1976). There may eventually be a third possibility. This was proposed by G. Jones (Hospital for Sick Children, Toronto), who demonstrated that using ultraviolet detectors of sufficient sensitivity, high pressure liquid chromatography (HPLC) can be used to routinely assay vitamin D, 25-hydroxyvitamin-D (25(OH)D), and $1,25(OH)_2D$. Since there is no specific assay for vitamin D itself, and competitive protein binding assays only measure the concentration of an individual metabolite, there would seem to be definite advantages to HPLC for these routine estimations.

Levels of the metabolite 24,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$) found in various studies still prove difficult to interpret. In human volunteers with levels of plasma 25(OH)D in the range 6.0–36.0 $\mathrm{ng\,ml^{-1}}$, C. M. Taylor (University of Manchester) reported circulating levels of $24,25(OH)_2D_3$ within the range 0.3–3.6 $\mathrm{ng\,ml^{-1}}$, and the absence of this dihydroxy metabolite in anephric patients. J. G. Haddad (Jewish Hospital of St Louis, Missouri) however, reported circulating $24,25(OH)_2D$ levels 2–3 times those of Taylor's, and in addition, normal levels of the metabolite in sera from anephric patients. Since anephric patients are missing the kidney enzyme vitamin D 24-hydroxylase, Haddad suggests that significant extrarenal 24-hydroxylase was present in his subjects.

Additional evidence for an extrarenal 24-hydroxylase was provided by H. F. DeLuca (University of Wisconsin); his laboratory has observed the conversion of $1,25(OH)_2D_3$ to 1,24R,25-trihydroxycholecalciferol ($1,24R,25(OH)_3D_3$) *in vivo* in nephrectomised rats and chicks. There is still some scepticism concerning the physiological importance of the trihydroxy metabolite but DeLuca reported that it is present in sufficient quantity *in vivo* to be regarded as significant.

The active form $1,25(OH)_2D_3$ is rapidly accumulated in target tissues. In the gut, for example, it regulates the transcription of the calcium binding protein (CaBP) gene. R. H. Wasserman (Cornell University, Ithaca) reported that in chick intestine in steady-state conditions—such as adaptation to egg laying or calcium or phosphate deficiency—his previous data showed good correlation between CaBP concentrations and calcium absorption. More recent data, however, suggest that another 'vitamin D responsive factor' in addition to CaBP is required for vitamin-D stimulated calcium absorption.

Other 'vitamin D responsive factor(s)' have been observed in the brush border membrane of chick intestine. D. E. M. Lawson (Dunn Nutrition Laboratory, Cambridge) has observed at least two membrane proteins which are synthesised more rapidly than CaBP in response to $1,25(OH)_2D_3$. And H. Rasmussen (Yale University School of Medicine, Connecticut) reported changes in the fatty acid composition of the membrane phosphatidylcholine (PC) fraction in membranes isolated from vitamin-D deficient chicks treated with $1,25(OH)_2D_3$ compared with vitamin-D deficient controls. The changes observed in the PC fraction of the treated chicks—associated with an increased ability of the membrane to take up calcium—were an increase in the ω : 6 fatty acids linoleic and arachidonic, and a decrease in at least one of the ω : 3 series, docosahexaenoic acid.

The functions of the two dihydroxy metabolites 24,25(OH)$_2$D$_3$ and 25,26(OH)$_2$D$_3$ are still far from being resolved. J. A. Kanis (Nuffield Orthopaedic Centre, Oxford) suggested that 24,25(OH)$_2$D$_3$ may act directly on bone to increase mineralisation, on the basis of the observation that 1 μg d^{-1} of 24,25(OH)$_2$D$_3$ (R+S epimers) administered to an anephric patient with osteomalacia, increased intestinal calcium absorption and reduced plasma alkaline phosphatase, without causing an increase in plasma or urine calcium. These effects were not observed by A. M. Pierides (University of Newcastle upon Tyne) however, in patients receiving as much as 16 μg d^{-1} of 24,25(OH)$_2$D$_3$.

The most ignored dihydroxy metabolite of vitamin D is certainly 25,26(OH)$_2$D. Very little is known about its properties and its *in vivo* origin is completely unknown. That it is a significant metabolite in man at least, was clearly demonstrated by E. B. Mawer (University of Manchester). In vitamin D replete individuals the serum concentrations of 25,26(OH)$_2$D and 24,25(OH)$_2$D are similar. Whereas the production of 1,25(OH)$_2$D$_3$ and 24,25-(OH)$_2$D$_3$ are governed by parameters such as the state of vitamin D nutrition and serum calcium concentration, no such controls are evident for 25,26-(OH)$_2$D$_3$ synthesis.

The papers referred to were only a few of the highlights of this workshop. But they do indicate that the next decade should be fruitful. By the 1980s the functions and mechanisms of action of all the vitamin D metabolites should be known, and the importance of 1,25(OH)$_2$D in phosphate regulation should be determined. The answers to these questions should provide reliable therapeutic procedures for the treatment of clinical disorders such as the renal osteodystrophies and postmenopausal osteoporosis. ☐

Minicomputers for quantum chemists

from Graham Richards

QUANTUM chemists have always been major users of the most powerful computing facilities. Indeed, in recent years, the advances in the subject have followed the introduction of each new generation of machines and the capability of doing the best work has, to a large extent, been limited to those with access to major computing centres. As a result the subject has been dominated by a small number of groups and not used as a tool by more applied chemists to study or clarify their own research problems. The rapidly falling cost and increasing power of minicomputers should lead to a radical change if it were possible to perform the massive *ab initio* quantum mechanical calculations on minicomputers rather than giant number-crunchers. A pilot study which succeeds in getting the quart into a pint pot and studying genuinely interesting problems has been conducted in the Chemistry Department in Berkeley.

The question "are minicomputers suitable for large scale scientific computation?" was answered in the affirmative by Schaefer in a paper presented at the IEEE Computer Society Conference held in Washington in September 1975 and in the proceedings of that meeting the relative capabilities and suitability of a number of minicomputers are compared. Since that date memory prices have fallen and new developments such as high speed array processors make the use of the minicomputer a very attractive alternative to the use of expensive large machines.

Calculations may take of the order of 30 to 40 times as long on a minicomputer as they do on a large scientific machine. However, since the calculations may be run overnight and the machine can be operated by graduate students or research personnel rather than professional operators, it may be much cheaper to work in this way.

The molecular structure calculations which have been attempted using a minicomputer have not been limited to the study of simple electronic ground states of closed-shell molecules. The Berkeley group has studied the excited electronic states of both glyoxal and ketene (*J. Am. chem. Soc.* **98**, 401, 2689; 1976). For each of the molecules, some 20 excited states are included and details are given of geometries as well as excitation energies.

The same minicomputer provided the means of studying some fundamental chemical reactions with *ab initio* molecular orbital calculations. Two reactions involving methylene have formed the subject of articles in the *Journal of the American Chemical Society* (**98**, 1653, 3073; 1976). For the least-motion insertion reaction of methylene and hydrogen to yield methane the transition state geometry and activation energy were calculated and the abstraction reactions of triplet methylene with hydrogen and methane studied in the same way. For the methane abstraction

$$CH_2(^3B_1)+CH_4 \rightarrow CH_3+CH_3$$

the work implies that the reaction should not occur to a significant degree at room temperature contrary to the predictions of Dewar based on his semi-empirical MINDO calculations.

Although it is still early to be sure that the minicomputer will lead to a welcome change of life for quantum chemists it is striking just how wide is the range of problems tackled to date with the relatively cheap form of computer. Not only have quantum mechanical calculations been performed, but semiclassical computations, such as atomic scattering from solid surfaces, have also been studied in this way; an example being the scattering of helium and neon from tungsten, the work of Miller and his colleagues (*J. chem. Phys.* **64**, 45; 1976).

If quantum chemistry can be made inexpensive enough for chemists in experimental research laboratories to run their own calculations then the day may at last come when experimentalists can run their own calculations routinely. This will save the literature being over-burdened with details of computations which may be directly interesting only to a small audience, and will enable practical chemists, such as medicinal chemists, to reap the benefit of useful calculations without needing to persuade a theoretical group to take on their work. ☐

A hundred years ago

THE total expenditure on the new building at South Kensington for the reception of the Natural History Collections now in the British Museum is stated in the new Civil Service Estimates to have been 206,472*l*. up to September 30 last. A further sum of 36,650*l*. is required to carry on the works up to the end of the present financial year. This amount has been already voted. The proposed vote for the present financial year 1877–78 is 70,000*l*., leaving the amount of 1,878*l*. necessary to complete the building, the total estimate having been 395,000*l*. We may remark that it is not only in this country that a new Museum of Natural History is in progress. Both at Paris and at Berlin the present buildings for the National Museum are found to be too small, and large sums are to be appropriated to their reconstruction.

From *Nature* **15**, March 1, 382; 1877.

review article

The continental drift debate

F. J. Vine*

The controversy within the earth sciences over the hypothesis of continental drift has been unusually protracted. Here Professor Vine reviews the history of the debate as recorded in the pages of Nature.

THE name most commonly and correctly associated with the hypothesis of continental drift is that of the German meteorologist Alfred Wegener (1880–1930). Wegener's first article on the subject appeared in 1912 and the first edition of his book *Die Entstehung der Kontinente und Ozeane* (The Origin of Continents and Oceans) in 1915. Presumably because of the first World War and the lack of an English translation, a review of Wegener's book did not appear in *Nature* until 1922[1] by which time a second edition was available (published in 1920). It seems probable that this together with Wegener's article in *Discovery* in 1922[2], constituted the first widespread exposure of the "Wegener hypothesis" to the English-speaking world and it prompted a great deal of discussion at scientific meetings and correspondence in *Nature*.

It is perhaps instructive to ask why it was that geologists had paid little attention to the concept of continental drift before the publication of Wegener's book, for Wegener was certainly not the first to suggest that the continents were once together and subsequently drifted apart. Several contributors to *Nature* in the 1920s were at pains to point out that Wegener was anticipated by, among others, Oswald Fisher (1882), W. H. Pickering (1907), F. B. Taylor (1910) and H. A. Baker (1911); and A. A. Robb in 1930 (ref. 3) unearthed and reproduced the reconstruction of the continents around the Atlantic published by Antonio Snider in 1858. A particularly concise and authoritative account of the philosophical development of the concept before 1900 appeared in *Nature* in 1970[4]. It was written by N. A. Rupke who identified, I think, the main reason why geologists did not consider drift before the publication of Wegener's ideas. All the earlier advocates had associated drift with a catastrophic event. Even Taylor, who, quite reasonably, suggested that drift might explain the formation of the Atlantic and Indian oceans and the young fold mountain belts, invoked the origin of the Moon out of the Pacific in the mid-Cretaceous as the ultimate cause. Although the earliest geologists were well-versed in biblical catastrophism, the concept of 'uniformitarianism' or 'actualism', first advanced by James Hutton and Sir Charles Lyell in the early nineteenth century, gradually replaced this earlier philosophy so that by the turn of the century the invocation of any sort of catastrophic event to explain geological phenomena was something of an anathema to all right-minded geologists. Surprisingly perhaps, Wegener's hypothesis was the first to formulate the concept of drift within the framework of 'uniformitarianism', that is, by processes operating today which have operated slowly but inexorably throughout time.

In addition Wegener went further than anyone before in attempting to document the geological and geophysical evidence for drift, and to provide, albeit rather unsuccessfully, uniformitarian mechanisms whereby drift might be taking place. Wegener was attacked on all three counts, on the basis of the geological and geodetic evidence he invoked, and the mechanisms he suggested. The debate dragged on inconclusively for 30 yr until even the perspicacious Arthur Holmes was driven to write, in 1953[5] "I should confess that, despite appearances to the contrary, I have never succeeded in freeing myself from a nagging prejudice against continental drift; in my geological bones, so to speak, I feel the hypothesis to be a fantastic one". However, the end was in sight; the advent of new and independent evidence suggestive of drift, from palaeomagnetic studies, resuscitated the idea in the late fifties and early sixties, and subsequently the post-war investment in marine geology and geophysics paid off in the form of providing compelling evidence for sea-floor spreading and hence continental drift. By the late 1960s the vast majority of geologists and geophysicists were convinced that continental drift was a reality; it was as if "the dream of a great poet" (Termier's words in 1926) had come true.

The reviewers of the various editions of Wegener's book which appeared in the 1920s seem to have suffered from a mixture of shock, incredulity and even some initial enthusiasm on reading Wegener's heretical ideas. The reviewer of the second edition[1] refrains from passing judgement, but concludes that "The revolution in thought, if the theory is substantiated, may be expected to resemble the change in astronomical ideas at the time of Copernicus". It is interesting to note that J. Tuzo Wilson made precisely the same analogy 45 years later following the essential confirmation of Wegener's hypothesis[6]. Grenville Cole[7] in a somewhat flowery but careful review of the third edition of Wegener's book could not decide whether to be serious or frivolous in discussing it. On the whole he took it seriously, if only as a challenge, and in so doing made some very interesting points. Gregory's review of the English translation of the third edition by J. G. A. Skerl also makes interesting reading[8]. Gregory is obviously stimulated by the boldness of

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Wegener's ideas and maintains that they were being favourably received by geologists in general at that time, an impression which is not corroborated, however, on reading other items of this period. Gregory is preoccupied with a possible connection between Asia and North America across the Pacific which he deduces on palaeontological grounds. Although he finds this inconsistent with Wegener's theory he finally admits that "The original theory has undergone so many modifications and its author is so delightfully open-minded, that it may be changed to agree with a trans-Pacific land connexion". Indeed more than forty years later J. Tuzo Wilson made just such an accommodation by suggesting that a fragment of southeast Asia (Cascadia) was rafted across the Pacific and accreted on to the western margin of North America[8].

An early convert to Wegener's ideas, J. W. Evans, writing in 1923[9], made some rather pertinent observations on continental margins, predicted largely submerged microcontinents and emphasised the futility of fitting coastlines rather than shelf edges in making continental reconstructions. Such a geological *faux pas* was committed as recently as 1962 by Sir Harold Jeffreys[10]. In this letter Jeffreys maintained, as he had done previously, "that Wegener's alleged fit of South America into Africa is a misfit by about 15°. This is obvious on inspection of a globe". Cambridge legend has it that it was this assertion which prompted Sir Edward Bullard to make his well-known and much reproduced 'computer fit' of the shelf edges bordering the South Atlantic[11]. Bullard's fit was near perfect; Sir Harold, of course, had been referring to the coastlines. Bullard's fit is in fact virtually identical to that obtained nearly 10 yr earlier by S. W. Carey using an analogue technique[12].

Carey, Professor of Geology at Hobart, Tasmania, is one of a long line of southern hemisphere geologists who championed drift through thick and thin, largely on the basis of the geological record of the southern continents plus India which suggests that they formed a single super-continent—Gondwanaland—for a period of at least 200 Myr before 200 Myr ago. It is not surprising therefore to find that some of the items appearing in *Nature* in the 1920s[13] indicate a favourable reception for Wegener's ideas in South Africa. It is also significant that one of these reports mentions the South African geologist A. L. du Toit. Alexander du Toit became one of the greatest advocates of continental drift, effectively taking up the banner of drift following Wegener's untimely death on the Greenland ice cap in November 1930. Ultimately du Toit published his classic text on drift—*Our Wandering Continents*—in 1937: and it was briefly reviewed for *Nature* by E. B. Bailey in 1939[14].

Of the relevant items which were contributed to *Nature* during the inter-war period, two, I think, are gems: the report of a Princeton summer school, written by E. B. Bailey in 1927[15], and the review of the proceedings of the Symposium on Continental Drift, held in New York in 1926, written by Arthur Holmes in 1928[16]. Bailey's account of the "Princeton Summer School of Geology and Natural Resources" 1927, led and organised by the legendary R. M. Field, makes fascinating reading. He is obviously fresh from, and filled with enthusiasm by, his two traverses of the North American continent. The result is a very vivid account of, and, one suspects, some very profound comments on, North American geology. At one point in describing the geology of Pennsylvania he is so struck by its similarities with the British Isles and Europe that he exclaims "It is as if the Atlantic did not exist or, in other words, as if Wegener, after all were a true prophet".

Holmes's review relates to the proceedings of the famous, if not infamous, mid-year meeting of the American Association of Petroleum Geologists, held in New York during November 1926. The meeting was subtitled "a Symposium on the Origin of Movement of Land Masses, both Inter-Continental and Intra-Continental, as proposed by Alfred Wegener". Wegener himself was present and the North Americans in particular gave him what would be termed in their own parlance "a hard time". In fact it is often said, with relatively little exaggeration, that as a result of this meeting no North American geologist seriously considered the possibility of drift for the next 40 yr. However, this was not true of all European geologists and certainly not of southern hemisphere geologists, as noted above. Holmes himself was one of the European geologists who was not at all averse to drift but was concerned that many of the particular arguments which Wegener used did his cause more harm than good. He noted that "There is, indeed a distinct danger that the easy disproof of large sections of the Wegener hypotheses may be mistaken for a demonstration of the impossibility of continental drift as a geological process. The important issue is now not so much to prove Wegener wrong as to decide whether or not continental drift has occurred, and if so, how and when". With the advantage of hindsight this strikes one as being a very profound summary of the situation pertaining at the time, and indeed for the following forty years. Because of his own prejudice in favour of drift, Holmes had the insight to pick out some of the more constructive and enduring suggestions made at this symposium. Thus he alighted on the suggestion of van der Gracht, "that there may have been a pre-Carboniferous 'Atlantic' which was closed up by the Caledonian diastrophism" a concept which was to be formulated in much greater detail 40 yr later by Wilson[17] and Dewey[18]. Again taking his cue from van der Gracht he states his own preference for convection in the mantle as the driving mechanism for continental drift, this being apparent from his other writings at this time[19]. Both it seems were following the suggestion of Ampferer[20] and others. Holmes goes so far as to conclude that "Geophysics will not be in a position to deny continental drift until it has thoroughly explored the possibilities of convection or other currents in a highly viscous substratum, and the forces set up by the interaction of magnetic and electric fields".

Several correspondents, quite justifiably, doubted the accuracy of the geodetic measurements which, Wegener claimed, substantiated that drift is occurring at the present time[21]. It is interesting to note that only now are relatively new instruments such as the mekometer, and new techniques, such as satellite and lunar ranging, providing the accuracy required to make direct measurements of drift. Even accurate measurement and monitoring of *intra*-continental crustal movements had received scant attention until the institution of the international 'Geodynamics' project in 1970 and the increased interest in earthquake prediction of recent years. Other letters bring out the limitations of fossils as indicators of continental drift and the inconclusive and often conflicting nature of the evidence provided by palaeontology[22].

It is perhaps not surprising therefore to learn that very little was written about the possibility of continental drift between 1930 and 1960, that is, following the initial discussion in the wake of Wegener's book, and before the revival of the debate following the palaeomagnetic discoveries of the late 1950s. It is also noticeable, and probably significant, that the first article on continental drift to appear in *Nature* is dated 1960. Before this all the contributions were reviews, reports, letters, presidential addresses or accounts of special lectures or discussions. Clearly this could be a result of editorial policy but in fact it is true of the geological literature as a whole during this period. It is as though no self-respecting geologist in the Northern Hemisphere was prepared to risk his reputation by publishing a full-length article on continental drift.

We are not, however, left completely without written records during these years; in fact two very useful summaries of the status of the debate were given by Watts in 1935[23] and Good in 1950[24], of which the latter is considerably less objective than the former. Perhaps one of the most pertinent statements made in this period was that of Sir Thomas Holland commenting on du Toit's demonstration of the remarkable similarities between the geology of Africa and South America from Lower Palaeozoic to Upper Mesozoic times: "If it be true that these continental masses have moved horizontally in late geological times away from one another, there is no reason why others should not have done so too, in spite of the circumstance that no completely satisfactory explanation of the mechanics involved can be offered at present. Further work on the lines so ably undertaken by du Toit is now more necessary than mathematical criticism with insufficient quantitative data regarding the physical state of the Earth's sub-crust under the continents and oceans"[25].

Towards the end of the period 1930–1960 it was often said that southern hemisphere geologists found it necessary to invoke drift because of the geological record on their continents but that northern hemisphere geologists found the geological evidence for drift equivocal. Even Holmes seemed to lose some of his initial enthusiasm for drift as is apparent in the review written in 1953 (ref. 5). I always found this very puzzling in that it seemed to me that the evidence for the former continuity and juxtaposition of structures, faunal provinces and even lithologies across the North Atlantic is at least as compelling as that for the reassembly of Gondwanaland. It came as no surprise to me therefore to find that British geologists such as Bailey[15] and Trueman[26] had been saying this as much as 30 yr earlier but that their witness had largely gone unheeded. It was as though most geologists and geophysicists considered drift to be disproved because of the inadequacy of the geodetic data and mechanisms that Wegener appealed to, and the unacceptable historical association with catastrophism which was perpetuated, unfortunately, by certain writers, well into the 20s and 30s. Taylor, for example, in 1926 (ref. 27) modified his ideas by postulating the capture of the Moon in mid-Cretaceous time rather than its origin out of the Pacific but this was still too catastrophic and non-uniformitarian to satisfy the geologists[16].

In spite of the fact that most geologists by the mid-1950s had rejected drift on the basis of geophysical and philosophical arguments, many geologists had from the outset acknowledged that the ultimate test of drift must come from the empirical evidence, that is, the domain of geology[28]. In fact, in the event, it was very empirical data which convinced most Earth scientists of the validity of continental drift, but data which derived from a marriage of geology and geophysics. Fundamental to these new discoveries was the development of the science of palaeomagnetism, the study of the intensity and direction of the Earth's magnetic field in the past as recorded in the permanent magnetism of rocks. If it is assumed that the Earth's magnetic field was essentially dipolar in the past, as it is today, then it is possible to test the hypothesis that the continents have changed their relative positions (except in the unlikely event that all relative movement has been along lines of magnetic latitude). If one makes the additional assumption that the magnetic field has been axially dipolar in the past as it is thought to have been recently, when averaged over thousands or tens of thousands of years, then clearly one can deduce the palaeolatitude of a particular continental area at any given time, and its orientation with respect to the meridian. Essentially, palaeomagnetic results confirm the hypothesis of continental drift as envisaged by Wegener and in particular vindicate the palaeolatitudes he deduced, on palaeoclimatic grounds, for his supercontinent of Pangaea during Permo-Carboniferous times[29]. Detailed palaeogeographies and palaeolatitudes for particular times, however, are still somewhat equivocal because of the limitations of the method. As Sir Edward Bullard is reputed to have said, "There is nothing in this field which could not be solved by palaeomagnetism if the results were ten times more numerous and the method ten times more accurate".

The decade of the 1960s also saw the confirmation of drift in terms of a sudden and spectacular advance in our understanding of the origin and evolution of the ocean basins. The theory which set the stage for this breakthrough first appeared in print in the pages of *Nature* in 1961 (ref. 30). It was presented by R. S. Dietz who termed it "sea-floor spreading", but the idea was originally conceived by H. H. Hess, as Dietz subsequently acknowledged[31]. Hess's original article was circulated as a preprint in 1960 and finally appeared in print in 1962 (ref. 32). Subsequently two important riders were added to the spreading sea floor hypothesis by Vine and Matthews in 1963 (ref. 33) and by Wilson in 1965 (ref. 34). The first proffered an explanation of the pronounced and linear oceanic magnetic anomalies and the second an explanation of the abrupt offset of mid-ocean ridge crests along transverse fracture zones. Both were later substantiated; the Vine and Matthews hypothesis as a result of the definition of a geomagnetic reversal time-scale and the acquisition of more magnetic anomaly data, and the Wilson "transform fault" hypothesis as a result of improved epicentral locations and focal mechanism solutions for earthquakes occurring on the oceanic fracture zones.

It is probably true to say that for most uncommitted geologists and geophysicists the most compelling and decisive evidence in favour of continental drift was provided by the data confirming the Vine–Matthews hypothesis, which were presented by Pitman and Heirtzler[35] and Vine[36] in 1966. The first public presentation of these results occurred at a conference in New York in November 1966, 40 yr to the month after the fateful meeting in New York in 1926. At this meeting also, L. R. Sykes presented the new seismological evidence which provided striking confirmation of Wilson's "transform-fault" hypothesis[37], thus providing additional confirmation of the hypotheses of spreading and drift. Soon after this both hypotheses were reformulated by McKenzie and Parker[38] and Morgan[39] in terms which became known as "plate tectonics". The concept of plate tectonics is itself an outgrowth of Wilson's transform fault hypothesis and the remarkable success of continental fitting at shelf edges which implies long term rigidity of vast areas of the crust and uppermost mantle.

In spite of the fact that the continental drift debate was not settled to most peoples' satisfaction until 1966 or 1967, most articles which appeared in *Nature* from 1960 onwards are favourably disposed towards it. This is not so representative of the geological literature as a whole during this period but probably reflects the fact that most of the initial palaeomagnetic results were obtained in British laboratories and as a result of this many British geophysicists were converted to belief in the theory. In addition, *Nature* seemed to attract many of the classic articles which paved the way for the ultimate proof and acceptance of the theory.

An article by Wilson in 1960 (ref. 40) provided an interesting curtain raiser to this final chapter of the continental drift debate. This article is doubly interesting because before this Wilson was in no sense a convert to drift and yet in this 'transitional' article one can see the beginnings of ideas which eventually amounted to sea-floor spreading and plate tectonics. He does this by allowing a certain amount of expansion of the Earth to account for the mid-ocean ridges. Other authors have favoured much larger scale

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expansion on the assumption that the Earth was originally entirely covered by continental crust[41]. This would involve a fivefold increase in volume, either since the Earth was formed or since drift was initiated. Such an idea has also been championed by S. W. Carey and B. C. Heezen. It has roots which go back to the mid-nineteenth century as mentioned by Rupke[4], but has never gained wide acceptance. With the advent and success of plate tectonics it now seems unnecessary and inappropriate to invoke large-scale expansion, although it has recently been revived yet again by H. G. Owen[42,43].

Despite the compelling evidence which has corroborated sea-floor spreading and continental drift within the past decade certain fundamental problems regarding the detailed reconstruction of Gondwanaland have persisted. These are, notably, the position of Madagascar against Africa[44] and whether parts of south-east Asia should be included in Gondwana[45,46]. In time, presumably, both problems will be solved by further palaeomagnetic studies, and indeed this stage may have been reached with respect to Madagascar, recent palaeomagnetic results favouring a southward motion relative to Africa as originally suggested by du Toit[47,48]. The question of the driving mechanism for drift and plate tectonics is also equivocal although some stimulating ideas have been derived in recent years from seismic studies of lateral heterogeneity in the mantle[49,50] and consideration of 'absolute' as opposed to relative plate motions[51,52].

I think it will be apparent from the foregoing that as regards the resolution of the continental drift debate, which was clearly fundamental to so many aspects of the science, geology stood still for essentially 40 yr, mainly it seems because geologists attached greater importance to the physical arguments rather than the geological evidence. It was as though the following remark of Eddington in 1922 fell on deaf ears, for it could equally well have been said in 1962.

"The time has gone by when the physicist prescribed dictatorially what theories the geologist might be permitted to consider". A. S. Eddington in an address to the Geological Society of London, 21st November 1922 (ref. 53).

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articles

Shape and pattern specification during microtubule bundle assembly

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The ways in which control of microtubule packing, linkage, and nucleation of tubule assembly, may be related to the cross-sectional shaping of developing microtubule bundles are explored.

In certain types of microtubule bundles the tubules are packed and linked together in precisely defined configurations. Many of these bundles are called axonemes (axle-threads) because they run along the central longitudinal axes of slender cell processes such as cilia, flagella, sperm tails, suctorian tentacles, and the axopodia of certain rhizopods[1]. On the other hand, highly ordered microtubular axostyles and nemadesmata are situated in the cell bodies of certain flagellates and ciliates[2]. Ciliary and flagellar axonemes with the well-known nine-plus-two microtubule configuration occur in a wide range of eukaryotic organisms and cell types. All the other types of highly ordered microtubule bundles so far described have only been found in certain unicellular 'organisms' such as protozoans, phytoflagellates, and the spermatozoa of a few arthropods[1,3,4].

These microtubule bundles are particularly suitable cell components for analysing control of growth and form at both the molecular and ultrastructural levels. The tubules are often packed in simple repeating patterns and the shapes and dimensions of microtubule bundles are sometimes specified with considerable exactness. Microtubule bundles are two or three orders of magnitude larger than viruses but the highly ordered architecture of most tubule bundles seems to result, like that of certain virus coats[5], from the self-assembly of large numbers of protein sub-units although only a small number of protein species are involved. Some of the control processes operating during the morphogenesis of microtubule bundles are likely to be similar to those concerned with the deployment of less compact and well ordered microtubule arrays Such arrays are an important feature of the organisation of most eukaryotic cells and are associated with various fundamental cell activities, including anaphase chromosome movement, hormone secretion, axonal and certain other forms of intracellular transport, formation of plant cell walls, and cell shaping and locomotion[6–10].

Does assembly proceed along basically similar lines for all microtubule bundles? The following account considers control of assembly for several types of bundles and provides some indication of the range of assembly procedures which are likely to operate It also indicates that construction of different types of bundles involves different combinations of assembly procedures For example, prefabrication of non-microtubular structures which act as templates seems to be required for direction of early stages in the assembly of some, but not all, types of microtubule bundles.

Nucleation of tubule assembly

Two closely related[11] (molecular weight about 55,000) protein sub-units, α and β tubulin, self-assemble in certain conditions to form microtubules[12,13] The tubules of a microtubule bundle almost invariably start to assemble in very close proximity to each other in a particular cell region Sometimes the number of tubules which start to assemble is specified exactly[14]. These observations imply that initiation of the assembly of each tubule of a bundle is nucleated *in vivo* by a special non-diffusible 'nucleating element' which is located in a particular cytoplasmic region If this were not the case, the substantial pools of tubulin which are present in some cells before tubule assembly starts[15–19] should almost always polymerise to form variable numbers of randomly oriented tubules, and rarely form tubule bundles with well defined numbers of closely adjacent tubules oriented parallel to each other The molecular nature of nucleating elements is unknown They differ in at least some detail from the ring-shaped tubulin aggregates which nucleate tubule assembly *in vitro*[20–22] because these aggregates can assemble from freely diffusible components. It is possible that the aggregates nucleate assembly of bundle tubules in *in vivo* conditions if they associate with certain non-diffusible components. If this is the case, it is the latter which represent the effective nucleating elements *in vivo*

Cytoplasmic localisation of bundle tubule assembly is achieved because the nucleating elements are usually bound together and may be attached to the surface of some other cell component[23–25] Concentrated groupings of nucleating elements and materials which connect them together have been termed microtubule-organising-centres (MTOCs) (ref. 26), nucleating sites, nucleating centres, and initiating sites[27] We have adopted the term MTOC.

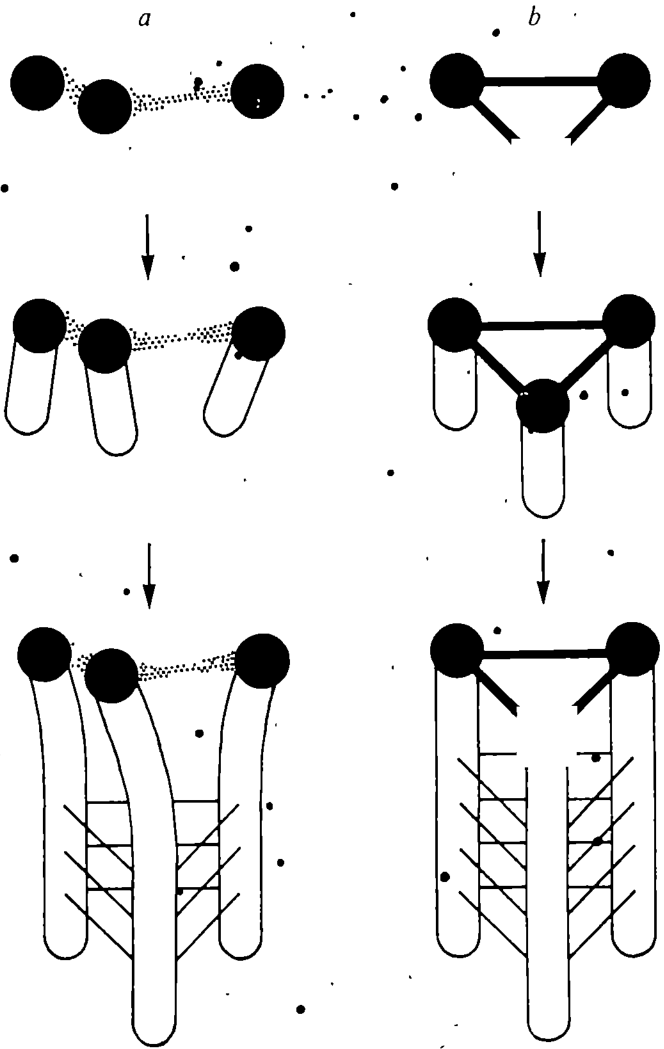
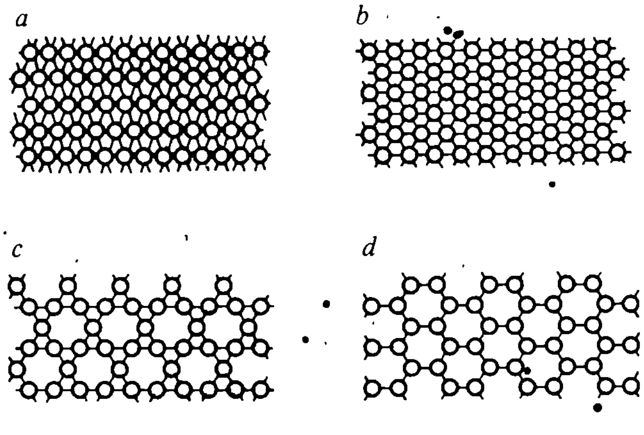


Fig. 2 Establishment of microtubule packing *a*, The nucleating elements (black spheres) are not precisely arranged Microtubules self-link into a regularly spaced arrangement *b*, The nucleating elements are bound together to form an MTNT which defines the arrangement of the tubules before they are connected together by links

Fig. 1 Equivalent patterns of tubule packing and linkage as revealed in cross-sections of tubule bundles. *a*, Nemadesma in the flagellate *Entosiphon*[30], *b*, nemadesma in the ciliate *Nassula*[28], *c*, axoneme in the radiolarian *Sticholonche*[31]; *d*, axoneme in the heliozoan *Raphidiophrys*[24]



Establishment of pattern

Patterns of microtubule packing in tubule bundles seem to be established in two main ways[9,24,28] For some bundles, pattern seems to be defined by self-linkage[9,28] when intertubule links connect adjacent tubules together. Links are usually peg-shaped structures with lengths of up to 40 nm and diameters of 3–7 nm. They are probably made up of protein subunits. The nexin-links of cilia are largely composed of protein subunits (molecular weight 150,000)[29], but no other details of the molecular composition of any other types of links are available. In several types of microtubule bundles most of the tubules are linked together in an equivalent fashion so that a repeating lattice of tubules and links is apparent when cross sections of the bundles are examined (Fig. 1). Such patterns may be established by self-linkage (Fig. 2*a*). This could be the case provided that the lengths of the links, and the positions and orientations at which they bind to tubule walls are precisely specified. At least some of these specifications are made in certain instances[28,32,33]. Analysis of axoneme assembly in certain heliozoans reveals that the pattern of tubule packing is established after nucleation of tubule assembly in a way which indicates that self-linkage is responsible[24,34].

A distinctly different procedure seems to establish pattern during assembly of certain other bundles. Pattern seems to be determined by a pre-existing arrangement of nucleating elements before tubule assembly begins because tubules are precisely positioned when they first start to assemble[14,28,35]. An MTOC in which nucleating elements are bound together in a highly ordered arrangement before they initiate tubule assembly is termed a microtubule-nucleating-template (MTNT) (ref. 28). There is no reason why such a template should not self-assemble from proteins coded for by chromosomal genes in the absence of any pre-existing copies of the template; these templates need not be regarded as genetically autonomous self-replicating entities. Although the pattern of microtubule packing is apparently maintained by self-linkage as tubules grow out from an MTNT (Fig. 2*b*), there may be advantages in assembling an MTNT to establish certain types of microtubule patterns and these are considered in the sections which follow.

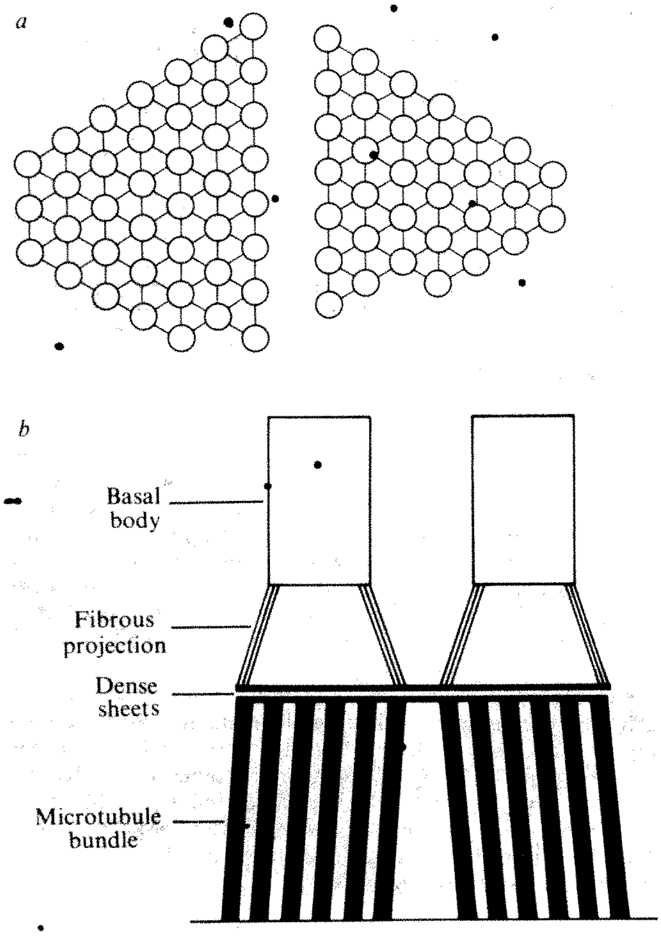


Fig. 3 The paired microtubule bundles of the ciliate *Urotricha*. *a*, Tubule arrangement and cross-sectional shape of a bundle pair based on micrographs of *U. faurei*[36]. *b*, Lateral view of the upper portions of a pair of bundles in *U. ovata*[37] showing attachment to basal bodies.

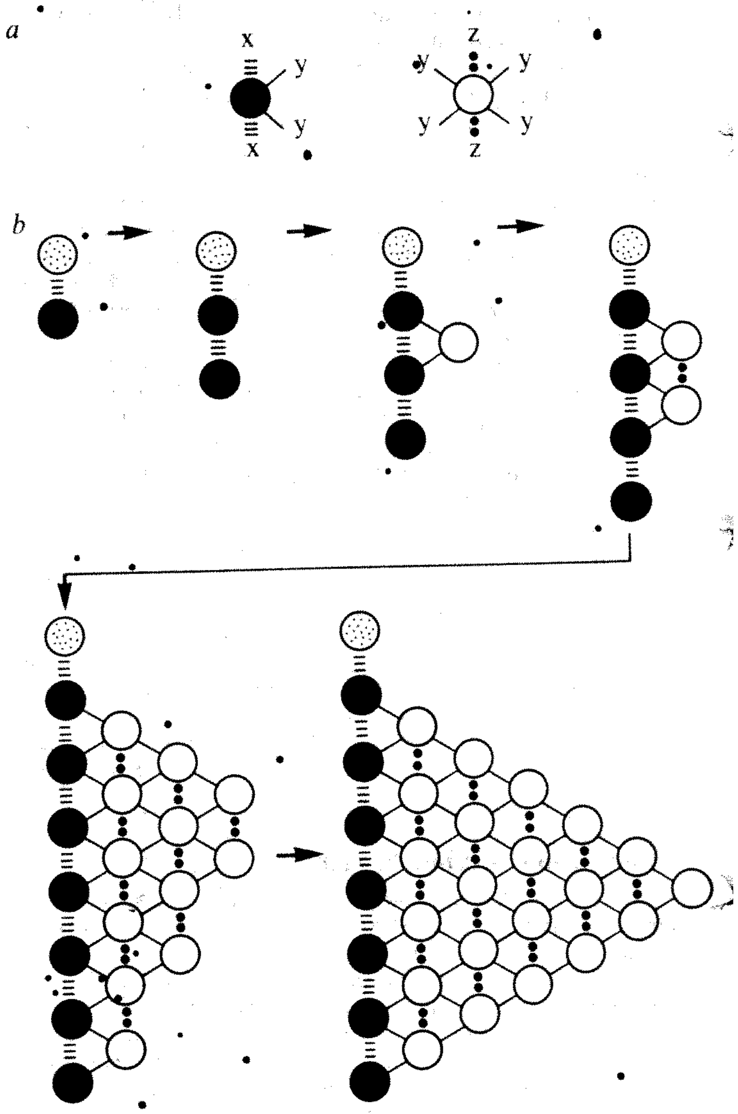


Fig. 4 A model scheme for assembly of a triangular MTNT. *a*, Schematic representation of the two types of nucleating elements required showing the arrangement of their binding points which lie in the same plane and can only bind to points of the same type on other elements. *b*, Stages in the assembly of elements to form the MTNT on a basal body projection. Each basal body which is attached to the top of a bundle has a projection (stippled) which bears an x binding point. An x bond can form between a basal body projection and any black element which is not already bound to any other element, and can also join two black elements provided one of the elements is already bound to another black element or a basal body projection. The y bonds can only join unbound or 'free' white elements to two other elements (black or white) which are already bound together so that two y bonds can form simultaneously when binding is effected. A z bond forms when both of the white elements it is to connect are already joined to the template by y bonds.

Triangular shape specification

The cytopharyngeal microtubule bundles (nemadesmata) of certain ciliates have rather precisely defined cross-sectional shapes although their tubules are apparently linked together in an equivalent hexagonal fashion. For example, in *Urotricha* the bundles have cross-sectional profiles which closely approximate to equilateral triangles in shape. Furthermore, bundles are closely juxtaposed in pairs and their cross-sectional profiles are precisely oriented with respect to each other (Fig. 3*a*). The tops of each of the bundles in a pair are attached to two sheets of dense material which are connected by fibrous projections to the proximal ends of a pair of basal bodies (Fig. 3*b*). No information is available about the sequence of events which occurs during bundle morphogenesis in *Urotricha* but in related ciliates the tubules of similar bundles grow out from dense sheets attached to basal bodies[25,38].

Specification of the cross-sectional shape and orientation of a bundle is most readily accounted for in theory by assuming that an MTNT forms by the assembly of two types of nucleating elements. If the conditions outlined in Fig. 4 are satisfied assembly of a triangular template will proceed along the lines indicated. The steric conformation of the elements and their binding points must be such that binding always results in all y bonds projecting from the same side along the row of black elements, and such that the points on the surfaces of the elements where nucleation of tubule assembly starts are all facing in the same direction (perpendicular to the lower surface of the template which faces away from the basal body). If the number of black elements available for assembly is limited, this number will largely determine the size of the template even when an excess of white elements is present. In these conditions the assembly scheme is a closed one like that for the coats of icosahedral viruses[39]. The orientation of the cross-sectional profiles of adjacent bundles with respect to each other may be determined by pre-existing spatial information in the form of precise orientation of the two basal bodies and their projections. There is considerable evidence that adjacent basal bodies in the ciliate cortex are often oriented with this degree of precision[40].

An important feature of the use of an MTNT in the situation described above is that all the bundle tubules can be identical. Each tubule would be potentially capable of linking to six neighbours hexagonally arranged around it. The extent to which this potentiality is realised depends on the number of neighbours specified by the MTNT. The possibility that triangular shaping could be established by self-linkage of two types of tubules along the lines suggested above for nucleating elements cannot, however, be excluded. Nevertheless, it is difficult to see how two types of tubules bound to sheets of dense material could have sufficient freedom of movement to self-link and form adjacent bundles with triangular cross sections oriented with respect to each other, unless tubule positions are specified fairly exactly by an MTNT at the start of tubule assembly.

Hollow axonemes with circular cross sections

The circular arrangement of microtubules in the axonemes of cilia and flagella is established at the beginning of basal body morphogenesis when exactly nine tubule triplets start to assemble in a regular circular arrangement. In *Paramecium* the sequence shown in Fig. 5 seems to occur. The dense disk may be an MTNT with nucleating elements bound in an evenly spaced circular arrangement around its perimeter. It is possible, however, that links can act as nucleating elements in this and certain other situations. When, and if this ever occurs, unlike a self-linkage procedure, tubule position is determined when tubule assembly is nucleated. For example, there may be only one nucleating element on the disk. Each of the links may be able to bind at one end at a particular orientation to certain points on existing A tubules and when it has done so nucleate assembly of a new tubule at its other end. Such a scheme accounts for the sequence of basal body tubule assembly which also occurs in several other organisms besides *Paramecium* (ref. 42). In some of these other organisms, however, a cartwheel structure with a hub and nine evenly spaced radial spokes is apparent before any of the tubules can be detected. These cartwheels may act as MTNTs if the tip of each spoke nucleates assembly of a tubule triplet[43,44]. Tubules and/or nucleating elements also seem to become grouped around a central non-microtubular structure during initial stages in the assembly of some other types of hollow axonemes which are circular in cross section. For example, when assembly of tentacle axonemes in the suctorian *Choanophrya* begins, tubules take up positions around a cylindrical 'condensation centre'[45]. This centre breaks down later in axoneme development when the circular tubule array is complete and tubules are joined together by links.

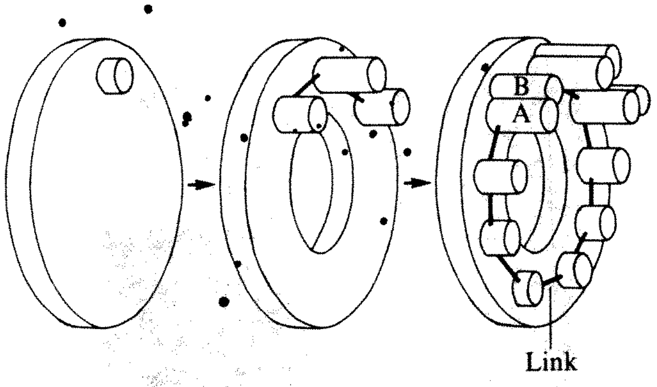


Fig. 5 Schematic diagram showing early stages in basal body assembly in *Paramecium* (ref. 41). One of the A tubules of a basal body starts to assemble shortly before the other eight. A second and third A tubule then assemble on either side of the first one and this sequence continues until a closed circular array has formed. Links connect tubules together at an early stage of their assembly and disappear shortly after the circular array is complete. The tubules grow out from a flat dense structure which is initially disk-shaped and then becomes hollowed out at its centre. Addition of the B tubule components of each triplet to A tubules starts before all the A tubules have formed.

The arrangement adopted by microtubules in sperm tails of the coccid insect *Planococcus* provides an example of a situation in which a rather precise microtubule configuration may be generated simply by crowding tubules closely together around a cylindrical structure without the assembly of an MTNT or very precise control of tubule linkage. During outgrowth of sperm tail axonemes in this and certain other coccids, microtubules are closely packed (sometimes into an approximately hexagonal array) around a cylindrical core of chromatin and other dense material[46,47]. In *Planococcus* two concentric tube-shaped arrays of microtubules are arranged around the dense core. A curved sheet-like array is usually situated inside the completely tubular ones and the dense core is often slightly indented where this array lies against it. One way in which this arrangement could be established, in this case by a 'close packing effect', is shown in Fig. 6. Examination of other microtubule bundles also indicates that patterns of microtubule packing which are hexagonal or nearly hexagonal, and certain types of dislocations in such arrays, may be established and/or maintained by a close packing effect[28,48]. The origin and

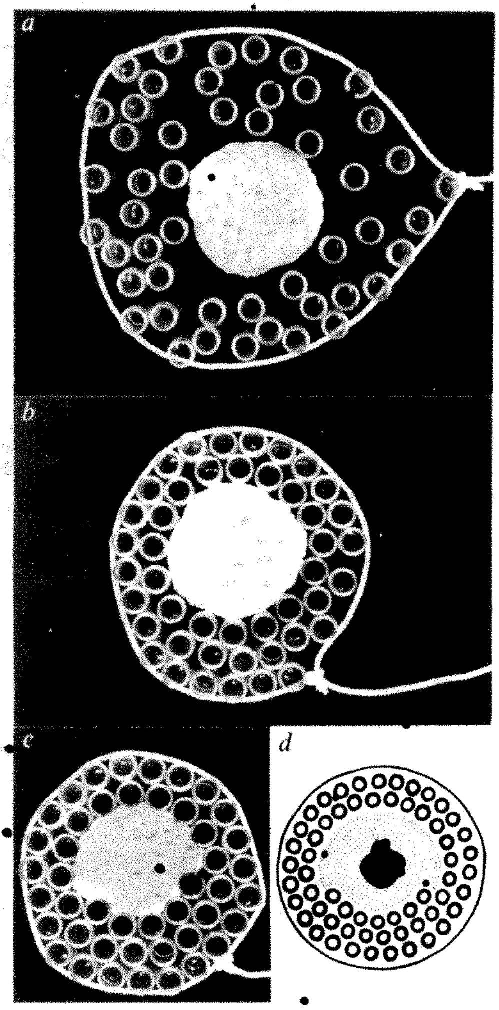


Fig. 6 Microtubule positioning in sperm of the coccid insect *Planococcus citri*. *a*, Dynamic model showing how tubules may be closely packed around a central cylinder of spongy material. Unstoppered glass specimen tubes stand on a smooth surface and are positioned fairly evenly around a cylinder of artificial sponge (the same height as the tubes). *b*, A string lariat is tightened and the tubes pack closely around the sponge. *c*, As tightening continues two circular arrays of tubes are formed and an inner curved sheet-like array presses into the sponge to produce an indentation like that in the dense core of the sperm. *d*, Diagrammatic cross section of a sperm based on electron micrographs[47]. Microtubules are arranged around a core of chromatin (black) and other dense material (stippled).

nature of the forces which might be involved in packing the tubules closely together in such situations are unknown.

Non-equivalent packing patterns

More complex events than any of those considered above may be involved during the assembly of microtubule bundles in which tubules are not all linked together in an identical fashion[8,34]. For example, in axonemes of centroaxoplastid radiolarians approximately two thirds of the tubules are linked to two neighbours, the others are linked to three[31]. In some such instances, the number of tubules in a bundle is exactly defined[49]. Descriptions of the assembly sequences for most bundles with 'non-equivalent' patterns of tubule linkage are not yet available. It is not clear whether all the tubules in these bundles are identical so that MTNTs are required to establish pattern, or whether pattern is generated when tubules of two or more types self-link. Greater freedom of movement will be required for tubules of two or more types to self-link than when only one type of tubule is involved. Can tubules of two or more types be confined in a localised region of cytoplasm and still have sufficient freedom of movement to effect self-linkage? A non-equivalent pattern of tubule packing seems to be established by self-linkage during generation of the double-spiral pattern in axonemes of the heliozoan *Echinosphaerium* (ref. 34). In this organism, however, tubules may be free to diffuse through the cytoplasm more extensively than is usually the case because the number, size, and spacing of axonemes does not seem to be very precisely defined. In addition, the suggestion[50] that relatively long-range allosteric interactions take place between adjacent tubules through their links during self-linkage raises the possibility that tubules which are initially identical can self-link in a non-equivalent fashion.

More information about the molecular composition of nucleating elements, intertubule links and MTOCs will facilitate further understanding of spatial control during assembly of microtubule bundles. Progress in this field is also impeded by our ignorance of the ways in which links bind to tubules. Whether they bind directly to tubulins or to other proteins[51–53] which may be bound to tubule walls is unknown. There are indications that in certain flagellar microtubules some of these proteins are mainly associated with particular protofilaments (rows of tubulins) along one side of each tubule[53]. Such unilateral protein arrangements may help to specify patterns of link attachment.

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Chemical modification of DNA polymerase phosphoprotein from avian myeloblastosis virus

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Fractionation of purified avian myeloblastosis virus DNA polymerase, after phosphorylation in vitro, *revealed the presence of a small acidic protein, a phosphate acceptor polypeptide with high specific activity. Its presence in the phosphorylated form with the polymerase resulted in as much as a 10-fold increase in the rate of DNA synthesis. Its presence in the dephosphorylated form with the polymerase had no effect in the rate of DNA synthesis.*

Since the recognition of an RNA-dependent DNA polymerase (RDDP) in RNA viruses[1-3], much effort has been expended on studies of its structure and function[1-11]. Two polypeptides, α and β, with molecular weights of 60,000 and 100,000, respectively[5-8], are generally demonstrable in preparations of RDDP from avian myeloblastosis virus (AMV). But the existence of the separate components and/or interrelationships between them remain obscure. The work reported here has revealed a constituent which appears in preparations of the highly purified RDDP activity isolated from AMV. This material has the properties of a nondialysable, small, acidic phosphoprotein, designated φ, which can be phosphorylated by a highly purified AMV protein kinase *in vitro*. Demonstration of φ is based on the separation of phosphorylated φ from purified AMV-RDDP by three different methods: (1) molecular sieving on Sephadex G-200; (2) isoelectric focusing on polyacrylamide gels, and (3) the use of sodium dodecyl sulphate–polyacrylamide gels. In addition, differences in the influence of phosphorylated and dephosphorylated φ on the rate of DNA synthesis by the polymerase are documented here. Finally, implications with respect to possible chemical modification of AMV-RDDP as a regulatory mechanism in the life cycle of the virion and its behaviour in the process of replication observed *in vitro* are discussed.

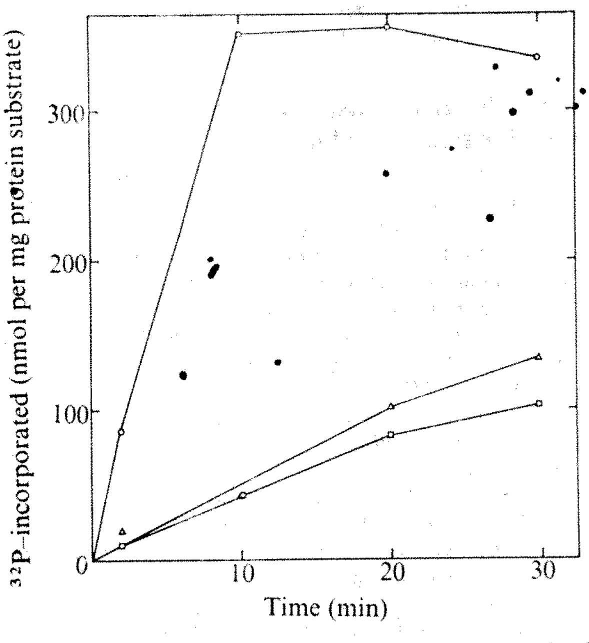

Fig. 1 AMV protein kinase-catalysed phosphorylation in the presence of γ-^{32}P-ATP and protein substrates of viral and non-viral origin. A 20-fold standard reaction mixture of AMV protein kinase was prepared containing 25 mM Tris-Cl (*p*H 8.3), 20 mM $MgCl_2$, 10 mM dithioerythritol (DTE), 20 mM KCl, 0.02% (v/v) Triton X-100, 0.2 mM γ-^{32}P-ATP (475 c.p.m. per pmol), and 20 µl of AMV protein kinase[12]. The amounts of phosphate acceptor protein per reaction were as follows: ○, 7 µg of purified AMV-RDDP, △, 200 µg of partially purified AMV-RDDP; □, 2,500 µg of α-casein (Sigma). The reaction was carried out at 35 °C. At the times indicated, 10-µl samples were placed on GF/C disks and treated as described before[21]. Radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer and converted to nmol of ^{32}P incorporated per mg of protein substrate.

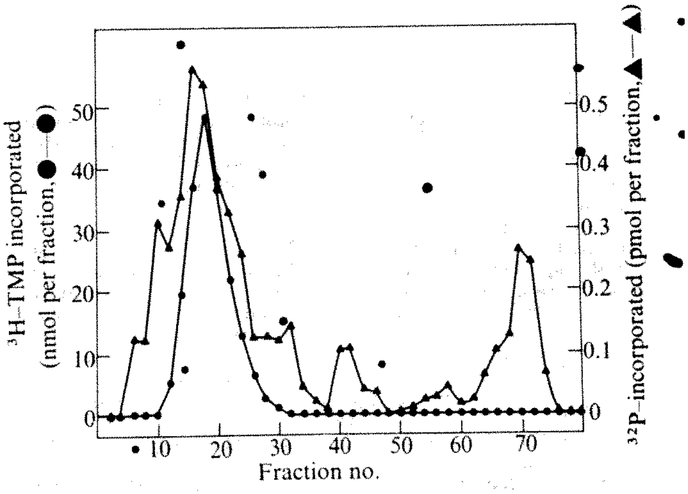

Fig. 2 Chromatographic analysis of AMV polymerase after phosphorylation with AMV kinase[12]. At the completion of the protein kinase reactions described in Fig. 1, all reaction products were dialysed against buffer A (200 mM potassium phosphate (*p*H 7.2), 0.1 mM EDTA, 2 mM DTT, 0.2% Triton X-100 containing 10% glycerol) for 36 h with four changes of buffer to remove γ-^{32}P-ATP. 0.5 ml (a fourth of the protein kinase reaction with purified AMV-RDDP as the phosphate acceptor) was subjected to chromatography by molecular sieving on Sephadex G-200 (1.6 × 60 cm) equilibrated with buffer A. Fractions of 1.8 ml were collected and analysed for ^{32}P-radioactivity (▲) as described in Fig. 1. RDDP activity (●) was determined after incubation for 60 min at 35 °C of a 10-µl sample with 50 µl of RDDP standard reaction mixture (50 mM Tris-HCl (*p*H 8.3), 0.5 mM ^{3}H-TTP (16.6 c.p.m. per pmol), 6 mM $MgCl_2$, 0.2 mM poly(A)$_n$·dT$_{12-18}$(10:1), 40 mM KCl, containing 100 µg of bovine serum albumin per ml), with 50-µl samples from each reaction placed on GF/C disks and treated as described before[21].

Phosphorylation of polypeptides and separation of φ

The kinetics of the phosphorylation reactions presented in Fig. 1 are expressed as incorporation of radioactivity from γ-^{32}P-ATP per mg of protein used as the phosphate acceptor in that reaction. All phosphorylation reactions described here

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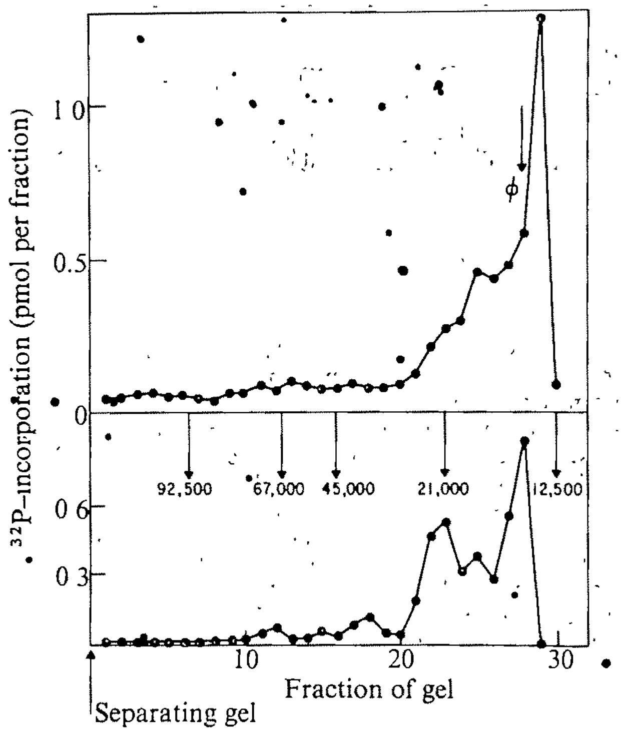

Fig. 3 Sodium dodecyl sulphate–polyacrylamide gel electrophoretic profiles of ^{32}P-radioactivity and standard proteins. Electrophoresis in the presence of 0.1% sodium dodecyl sulphate was carried out on columns (6 × 120 mm) of 15% polyacrylamide separating gel, including 6% polyacrylamide stacking gel as described by Laemmli[23] Protein samples were precipitated at 4 °C by addition of trichloroacetic (TCA) and (final concentration 10%), collected by centrifugation (20,000*g*, 20 min), washed with acetone and suspended in sample buffer. When β mercaptoethanol was added, samples were heated for 2 min to 100 °C to dissociate metastable aggregates and a 0.3-ml sample was applied to each polymerised gel. Electrophoresis was carried out at 60 V until the sample passed the stacking gel, and was then increased to 120 V for about 5 h at 18 °C. In gels intended for staining, the proteins were precipitated during 1 h at 60 °C with 14% TCA, and the gels were then washed at room temperature with 10%, 5% and 3% TCA within 24 h. These gels were then stained in a 0.05% solution of Coomassie brilliant blue R-250, in a mixture of methanol–acetic acid–water (45:9·46) during 2 h, followed by washing in the same solvent until the background was colourless. The gels were then placed in 9% acetic acid to reswell, after which they were photographed and/or traced Gels intended for measurement ^{32}P-radioactivity distribution, were sliced (collecting three slices per fraction) and solubilised, and radioactivity distribution determined as described before[24]. The molecular weight of AMV-polypeptides was calculated from their migration relative to proteins of known molecular weight, as described[25]. Upper graph, migration of ^{32}P-radioactivity distribution from an aliquot of IEF pool 1 (which focused with an average *p*I of about 4) Lower graph, standard proteins; phosphorylase α (92,500 daltons), bovine serum albumin (67,000 daltons), ovalbumin (45,000 daltons), ^{32}P-α casein (21,000 daltons) and cytochrome *c* (12,500 daltons). In addition, the distribution of ^{32}P-radioactivity from an aliquot of IEF pool 2 (which focused with an average *p*I of 7 0–7.5 in association with RDDP).

were carried out with our most purified fraction of AMV protein kinase[12].

On the basis of values of nmol of ^{32}P-incorporation per mg of protein substrate present in the non-viral and viral polypeptides examined, one of the virion polypeptides φ found in association with our most purified RDDP fraction displayed the greatest specific activity of phosphorylation (Fig 1 and ref. 12).

Fractionation, by molecular sieving on Sephadex G-200 (Fig. 2), of an aliquot from this protein kinase reaction, in the presence of our most purified RDDP (Fig. 1), followed by measurement of the distribution of RDDP activity, and ^{32}P-radioactivity, revealed that about 82% (Fig. 2 fractions 10–25) of the total ^{32}P-radioactivity on the column coincided with the RDDP activity. Most of the remaining ^{32}P-radioactivity eluted just behind myoglobin (fractions 65–75). Fractionation by isoelectric focusing on polyacrylamide gels[12] of another aliquot used for gel filtration as above, followed by measurements of the distribution of RDDP activity and ^{32}P-radioactivity, revealed that most of the ^{32}P-radioactivity on the gel focused with an average *p*I of about 4.0. The remaining ^{32}P-radioactivity on the gel focused with an average *p*I of 7.0–7.5 in association with RDDP (ref. 12).

Isoelectric focusing of the enzyme resulted in considerable losses of RDDP activity. Incomplete dissociation of φ protein from RDDP proteins is due to intramolecular salt link(s) between the phosphorylated (anionic) site(s) of φ and neighbouring basic (cationic) amino acid(s) of RDDP α and/or β polypeptides, since in high salt complete dissociation resulted[12]. Furthermore, in denaturing conditions (Fig. 3), complete dissociation of φ protein from α and β polypeptides was demonstrated (see later).

Electrophoresis of a sample identical to those used for gel filtration and isoelectric focusing revealed (Fig. 3) that all the ^{32}P-radioactivity migrated faster than α casein and slower than cytochrome *c* (Fig. 3, upper graph). No detectable radioactivity was observed in the regions of the gel where α and β polypeptides from purified RDDP would be expected with molecular weights of about 60,000 and 100,000 respectively. On the basis of the distance of migration of several marker proteins (Fig. 3, lower graph), the phosphoprotein (φ) found in association with purified RDDP was estimated to have a molecular weight of about 15,000. Although these results demonstrate association of φ with purified RDDP and complete dissociation of φ from RDDP, they do not exclude the possibility that φ is produced by limited proteolysis of the RDDP real enzyme (holoenzyme) at some time in the life cycle of virions and/or during purification[13-16].

Effect of *in vitro* phosphorylated and dephosphorylated φ

Purified AMV-RDDP was incubated for 30 min at 4 °C in the absence and presence of a preparation of purified phosphorylated ^{32}P-φ. Aliquots of each sample were then used to measure rates of ^{3}H-dGMP incorporation directed by poly(C)$_n$·dG$_{12-18}$ at the times indicated (Fig. 4*a*). In the presence of phosphorylated φ, the rate of poly(C)$_n$ dG$_{12-18}$-directed, ^{3}H-dGMP incorporation increased as much as 5–10-fold (Fig. 4*a*) Addition of larger amounts of phosphorylated φ, however, inhibited the rate of ^{3}H-dGMP incorporation (results not shown) When phosphorylated φ was added to purified AMV-RDDP reactions directed by poly(A)$_n$·dT$_{12-18}$ (Fig 4*b*) or AMV RNA·dT$_{12-18}$ (Fig. 4*c*), the rates of polymerisation increased but not as much.

Dephosphorylated φ was made as described in Fig. 5. Most purified AMV-RDDP was incubated for 30 min at 4 °C in the absence and presence of dephosphorylated φ together with the controls designated in Fig. 5. Removal of P$_i$ from φ eliminated the observed stimulatory effect of phosphorylated φ (Fig. 4). This is taken to mean that AMV phosphoprotein phosphatase has essentially completely dephosphorylated φ In other experiments on the dephosphorylation of φ with alkaline phosphatase or AMV phosphoprotein phosphatase, incomplete dephosphorylation was observed (results not shown). The influence of phosphorylated and dephosphorylated φ on the rate of acid solubilisation of ^{3}H-poly(A)$_n$ by purified AMV-RDDP from the hybrid homopolymer ^{3}H-poly(A)$_n$·poly(dT)$_n$ has been examined and the results will be reported elsewhere.

Implications

My results are relevant to several questions about the nature of RDDP. (1) What polypeptide(s) constitute the real enzyme? Reported results[7-9,15] support the notion that one of the two polypeptides (α) with a molecular weight of about 70,000 from

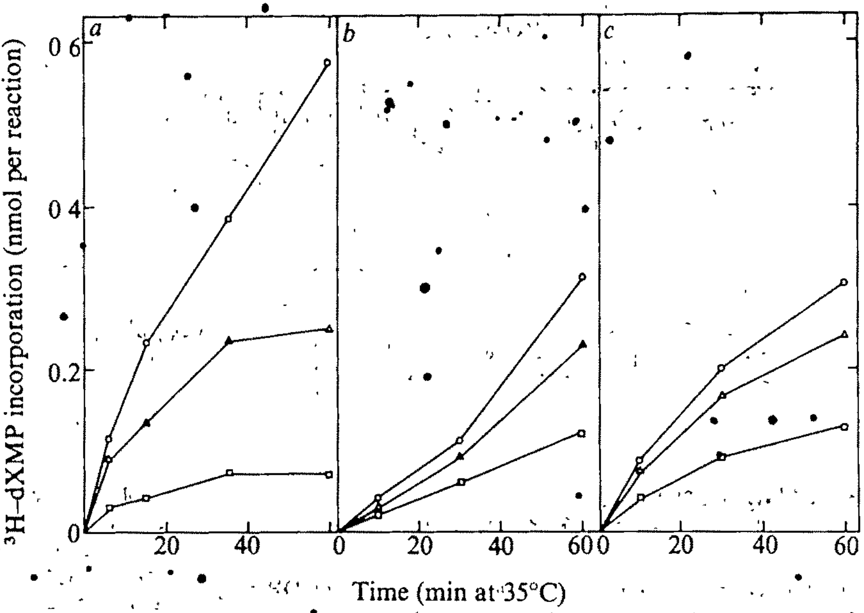

Fig. 4 Incorporation of radioactivity by a purified fraction of AMV-RDDP in the absence and presence of φ. 66 ng of the enzyme was incubated in the absence and presence of 2 µl and 5 µl of φ at 4 °C for 30 min, and all samples were made up to 12 µl with buffer A. After 30 min of pre-incubation of the above samples, 5-µl aliquots were added each to a 100 µl of the reaction mixtures described below and incubated at 35 °C for the time designated. *a*, ^{3}H-dGMP incorporation directed by 0.2 mM poly(C)$_n$. dG$_{12-18}$. *b*, ^{3}H-TMP incorporation directed by 0.2 mM poly(A)$_n$.dT$_{12-18}$. The homopolymers above were at a molar ratio of 10 adenosine to 1 thymine, and 10 cytidine to 1 deoxyguanosine, respectively. *c*, ^{3}H–TMP incorporation directed by 14.8 A_{260} AMV RNA per reaction in the presence of 0.2 mM dT$_{12-18}$ At the times indicated, 10-µl aliquots were placed on GF/C disks, treated, and the rates of ^{3}H-XMP incorporation were determined as described in Fig. 2. □, AMV-RDDP activity in the absence of φ, △, in presence of 2 µl φ; ○, in presence of 5µl of φ

AMV is a proteolytic degradation product[8,15,34], from (β) polypeptide which has a molecular weight of about 110,000. This possibility is supported by demonstrations that animal viruses contain proteinase activity involved in the cleavage of precursors of polypeptides (similar to those) found in the mature virions[13-16]. Specific proteolytic degradation has also been demonstrated with several DNA and RNA enzymes in prokaryotes[17-20]. (2) Why is the DNA product small[4-10]? Published reports[1-11], show that something was missing from the enzyme preparations used in previous studies. In 1972 Leis and Hurwitz described the isolation and characterisation of a heat labile, nondialysable factor from AMV that stimulated DNA synthesis, but no further studies have been reported. (3) Why does AMV-RDDP exhibit low fidelity when copying a variety of polynucleotide templates[11]? The high error rate of AMV-RDDP has been attributed to the nature of the polymerase[11]. Mizutani and Temin[34], however, have emphasised the importance of the reaction conditions in determining error-prone DNA polymerases in cell-free studies, and have suggested that changes in the physiological environment or altered DNA polymerases are responsible for the high mutation rates observed[11]. The results reported here support the suggestion that altered DNA polymerases in the life cycle of the virion and/or during purification are responsible for the high-infidelity observed[11,34]. This possibility can be tested by examining the influence of φ on the fidelity of AMV-RDDP enzyme in a cell-free system.

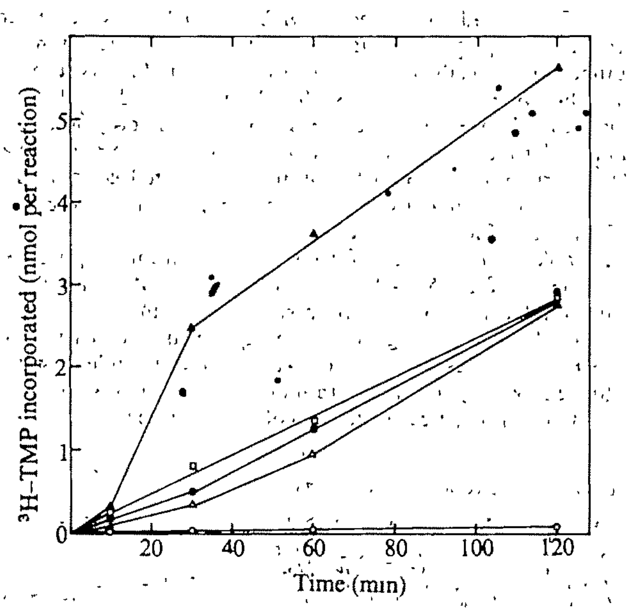

Fig. 5 Incorporation of radioactivity by a purified fraction of AMV-RDDP, in the absence and presence of phosphorylated and dephosphorylated φ. Dephosphorylated φ was made as described below. 3.5 µg of the enzyme was incubated for 30 min at 4 °C as indicated below, and all samples were made up to 60 µl with buffer A. After 30 min of pre-incubation, all samples were placed in ice and 10µl of each was added to 100 µl of the standard RDDP reaction mixture as described in Fig. 2, and incubated at 35 °C for the time designated. At the times indicated, 20-µl aliquots were placed on GF/C disks, treated, and the rates of ^{3}H-TMP incorporation were determined as described in Fig. 2. △, AMV-RDDP alone; ▲, AMV-RDDP in presence of ^{32}P–φ; □, AMV-RDDP in presence of dephosphorylated φ; ○, dephosphorylated φ alone; ●, AMV-RDDP in presence of bacterial alkaline phosphatase. Phosphorylated φ that had been dialysed to remove orthophosphate against Tris buffer was dephosphorylated with 20 µg of alkaline phosphatase in Tris-HCl (50 mM, *p*H 8.0) and MgCl$_2$ 5 mM). Dephosphorylation was carried out for 30 min at 35 °C. A sample equivalent to 5 µl of the phosphorylated φ and 5 µl of the phosphorylated φ were used in each case.

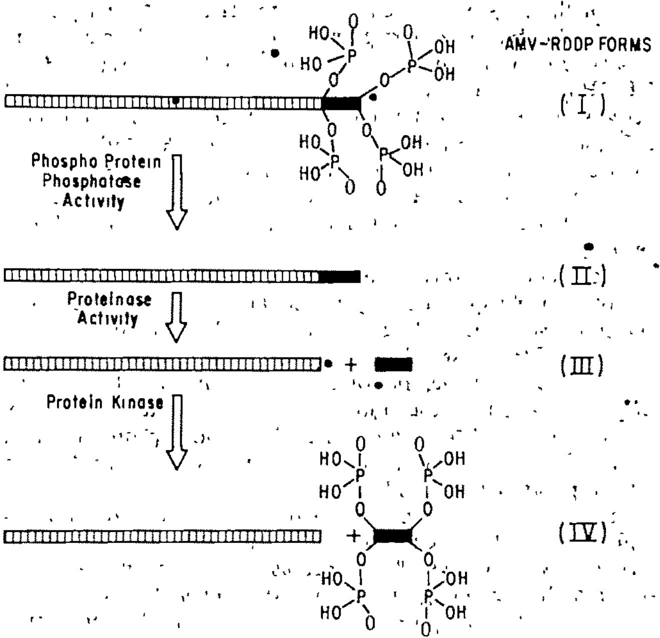

Fig. 6 Hypothetical model on the sequential changes in AMV-RDDP during its purification and/or at some time in the life cycle of the virion.

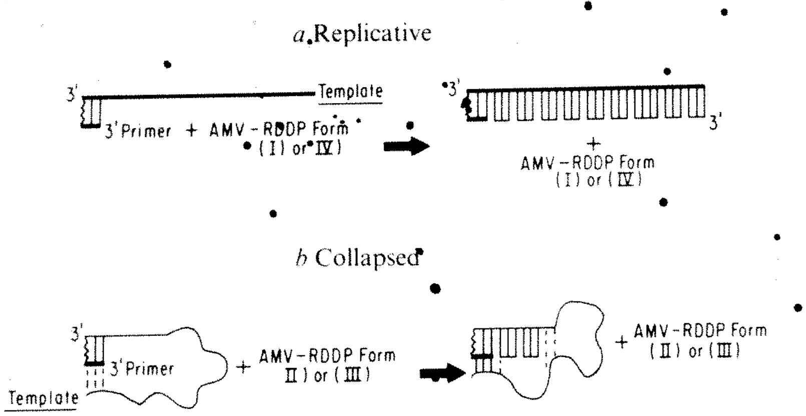
a. Replicative
3'
Template
3' Primer + AMV-RDDP Form (I) or (IV)
3'
3'
+
AMV-RDDP Form (I) or (IV)
b Collapsed
3'
3' Primer
Template
+ AMV-RDDP Form II) or (III)
+ AMV-RDDP Form (II) or (III)

Fig. 7 A model to account for the relatively small size (5–10S) and unfaithful nature of the products observed with AMV-RDDP and other oncornaviruses. *a*, Forms I and IV of AMV-RDDP (see Fig. 6) contain φ a large oligophosphoprotein, which helps to maintain the template structure in the replicative form during DNA synthesis. *b*, Forms II and III of AMV-RDDP (see Fig. 6) contain the dephosphorylated form of φ, which permits the replicative nature of the template to convert into the collapsed form, by means of intramolecular hydrogen bonding[26].

Model of possible enzymatic modification of RDDP

The model shown in Fig. 6 predicts that AMV-RDDP may exist in four forms at some time in the life cycle of the virion and/or during purification of the polymerase. This is facilitated by the combination of three enzyme activities—phosphoprotein phosphatase, proteinase and protein kinase. Association of these activities with purified virions from avian myeloblastosis has been established (refs 12, 13, 14, 16, 27 and unpublished results). Form IV of AMV-RDDP is strongly supported by the results reported here. φ is the phosphate acceptor protein with the highest specific activity in a purified AMV-RDDP preparation (Figs 1–3). Whether φ and AMV-RDDP are ever covalently linked (AMV-RDDP form I) remains to be seen. The existence of form I of AMV-RDDP is so attractive that it requires further experimentation. Demonstration of the existence of form I will assist in the documentation of the possible existence of forms II and III. The results presented in Fig. 5 support the existence of form III. In addition, I have observed a difference in the stability between AMV-RDDP activity in lysates buffered with phosphate (no loss of activity in 48 h at 4 C) and lysates buffered with Tris-HCl (76% loss). Furthermore, when such lysates were subjected to isoelectric focusing, specific polypeptides from lysates buffered with phosphate had fIs more acidic than those observed in lysates buffered with Tris-HCl (ref. 37 and unpublished results).

I conclude that this is due to the inhibition of AMV phosphoprotein phosphatase[12] by phosphate, a potent inhibitor of phosphatases. Depending on the procedures used to purify AMV-RDDP and whether or not throughout purification and storage of the enzyme, the solutions are buffered with Tris-HCl or phosphate buffer, the amount of phosphorylated φ may vary considerably and thus influence the yields, properties and form(s) of the enzyme isolated. There is support for this in several published reports[30–33,37].

In this respect it is interesting that in enzyme preparations buffered with Tris-HCl throughout purification only 10–20% recovery was observed[30,31], whereas in enzyme preparations buffered throughout purification with phosphate, as much as 70–90% recovery has been reported[32–33,37]. It is also interesting that studies *in vitro* on the infidelity of AMV-RDDP were conducted with enzyme preparations maintained throughout purification and stored in solutions buffered with Tris-HCl (ref. 11). Furthermore, dephosphorylation of purified AMV-RDDP with alkaline phosphatase from *E. coli* resulted in a decrease in the rate of polymerisation[37]. Similar results have been reported with RSV-RDDP (ref. 28).

I propose a model of AMV-RNA replication which may account for the small size (5–10S) of the products[4–10] and infidelity of replication of RDDP (ref. 11). This is represented diagramatically in Fig. 7*b*. RDDP will replicate only when the polymerase (forms II or III, Fig. 6) is present because φ is dephosphorylated in both these forms, so that the template exists in the collapsed form by means of intramolecular hydrogen bonding[26]. In contrast, Fig. 7*a* shows the replicative form of RNA in the presence of AMV-RDDP forms I or IV. This is made possible by the presence of the phosphorylated φ, which helps the template to remain so, while the polymerase makes the complete and faithful cDNA. One of the predictions in this model (Fig. 7*a*) has far-reaching implications concerning the size and fidelity of the polymerase product. In appropriate conditions, AMV-RDDP form IV, which can now be made available, may be able to make complete and faithful copies of the RNA from these virions.

The activity of RDDP from murine and simian C-type RNA viruses in the presence of phosphorylated φ increased by as much as 10-fold. No increase in the DNA polymerase activity from *E. coli* and *Mucrococcus luteus* could be detected in the presence of phosphorylated φ (C.M.T. in preparation). A comparable specificity of bacterial protein factors towards their homologous polymerase has been reported[38]. The pronounced enhancement observed on the rates of polymerisation catalysed by the polymerases from avian, murine and simian RNA viruses in the presence of phosphorylated φ, implicates φ as an important constituent in the replication of the genetic information of RNA viruses.

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Structure of an antibody combining site by magnetic resonance

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The structure of the combining site of the DNP binding IgA mouse myeloma protein MOPC 315 has been determined by a combination of high resolution nuclear magnetic resonance, electron spin resonance, model building and chemical modifications. This approach yields the general dimensions of the site, its polarity and asymmetry features, the assignment of the DNP-contact residues and their three-dimensional coordinates.

ONE of the central problems in immunochemistry is the structural basis of antibody specificity, how does such a high diversity in molecular recognition reside in what seems to be a family of proteins closely related in their chemical structure? Recent progress in X-ray analysis of Fab′ fragments of homogeneous myeloma proteins has led to the elucidation of the three-dimensional structure of the binding site of two antibodies, the human protein New which binds vitamin K and the mouse protein McPC 603 which binds phosphorlycholine[1,2]. From these studies it is clear that the residues forming the sites that are complementary to the antigen are contributed by the hypervariable regions[3] (three segments of 5–10 residues each, in both light and heavy chain) and that replacements of amino acid residues in these segments will generate binding sites with new specificities. Such replacement will not disturb the 'immunoglobulin fold' of the variable domains which remain similar in all antibodies. Thus on a background of a common three-dimensional structure a vast number of specificities can be generated. This diversity in recognition is the essence of the immune system and therefore a comparative analysis of a large number of combining sites is required in order to provide the details of molecular recognition by antibodies[4]. It is also important to develop new methodologies by which the structure of the antibody site can be analysed in solution and yield information comparable with that obtained from crystal studies We describe here an attempt to elucidate the structure of the binding site of the DNP binding protein 315, a mouse IgA myeloma protein[5], by a combination of high resolution nuclear magnetic resonance (n.m.r.), electron spin resonance (e.s r.), model building and chemical modification. We show that by this approach it is possible to determine the general dimensions of the site, its polarity and asymmetry features and to assign the contact residues with the ligand, as well as their three-dimensional coordinates.

Model building as a basis for structural analysis

A quantitative comparison of the tertiary structure of human and mouse variable domains has demonstrated that the immunoglobulin fold is very similar in different variable domains while structural differences occur predominantly in the hypervariable loops which comprise the combining site[6] Hence the variable region can be regarded as consisting of a rigid framework to which are attached the hypervariable loops This provides a basis for comparative analysis of antibody combining sites by model building, which then uses the coordinates of the framework from X-ray analysis data of known immunoglobulins and the sequence of the immunoglobulin which is to be analysed. Considering the diversity of immunoglobulins and the impracticality of X-ray analysis of all interesting antibodies this model building approach is an attractive way for a comparative study of antibody combining sites, provided the resulting combining site and the binding of ligand to it can be tested by various physical methods. Some of the features of the combining site of protein 315 have already been indicated by Poljak on the basis of comparison with the structural model of protein New (ref. 7). More recently, Padlan *et al.*[8], have described the model building of the site of protein 315 on the basis of coordinates derived mainly from protein 603 and using also information from protein New. The resulting combining site[8] is presented in Fig 1 and is the subject of further refinement on the basis of the n.m r. data presented in this article. The main feature of 315 combining site is its hydrophobic nature. The clustering of hydrophobic residues is more extensive than in either protein 603 or New. The middle of the surface produced by the hypervariable loops is dominated by a pronounced cavity which can accommodate the dinitrophenyl ligand and the depth of this cavity is approximately 1.2 mm. Details of this combining site are discussed by Padlan *et al*[8]. At first sight it seems that there are several different modes of binding that can accommodate the ligand in it We shall demonstrate both that the n.m.r. results dictate an unique arrangement of the DNP ligand in the cavity of the protein 315 and can be used to refine the three-dimensional positions of various residues in the site. We have also probed that part of the combining site, which binds the side chain of DNP ligand by using homologous series of spin-labelled haptens or alkyl phosphonate derivatives of haptens.

Gross architecture of the combining site

Since approximately 50% of the binding energy of DNP ligands to protein 315 is contributed by the hapten side chain[9], it is of great interest to study the environment of

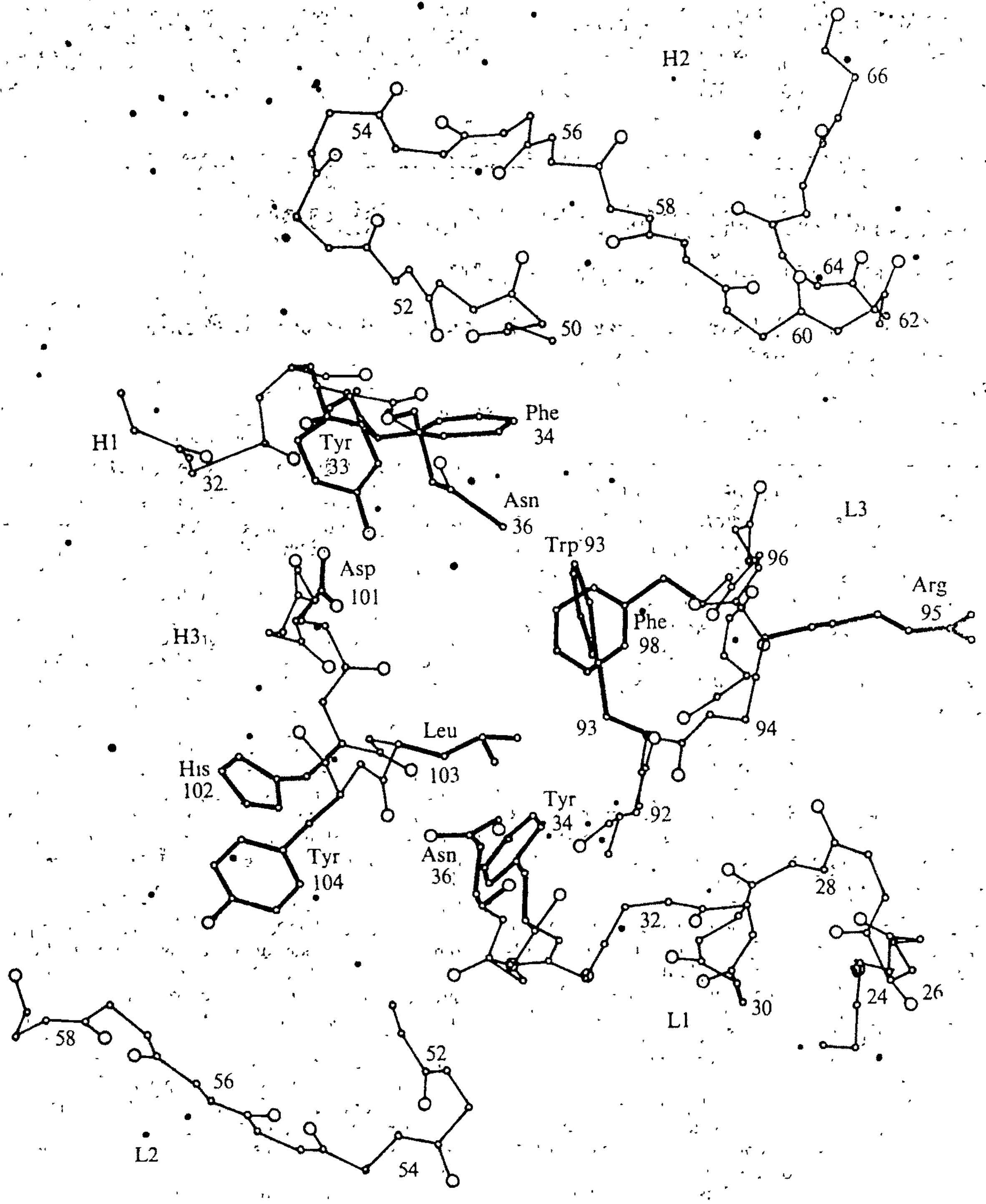
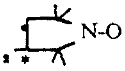
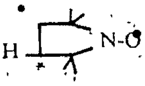


Fig. 1 The predicted combing site of MOPC 315 This has been obtained from the coordinates of Padlan *et al.*, which have been very kindly made available to us before publication. The hypervariable loops of the heavy (H) and light (L) chains and the hapten binding site are indicated.

the combining site in this area. To do this we have used two series of haptens of systematically increasing chain length with magnetic resonance probes attached. One series consists of either six- or five-membered nitroxide rings attached to the DNP moiety which is analysed by the e.s.r. spectra and the other series consists of alkyl phosphonate groups attached to the DNP moiety which are analysed by their phosphorus n.m.r. signals

The spin label mapping procedure involves comparison of the observed e.s.r. hyperfine splittings with those expected as a result of different postulated motions for the spin label. An indication of the type of analyses has been given previously for DNP ligands containing a six-membered nitroxide ring[10]. From that study, it emerged that the DNP ring is rigidly held in the combining site, that the depth of the site is 1.1–1.2 nm and that the minimum lateral dimensions at the entrance to the site are 0.6×0.9 nm. However, this method has now been extended to the analysis of five-membered spin-labelled haptens with chiral centres. Measurements of such haptens result in separate bound signals from each enantiomer[11] and this shows that the site is asymmetric with respect to the plane of the DNP ring. The two enantiomers which probe different sides of the site are restricted differently in their motions. Comparison of the experimentally measured values of the maximum hyperfine splittings, A_{max} with those calculated on the basis

Table 1 Deduction of lateral dimensions of the combining site by comparison of experimental values of the esr hyperfine splitting constants with those expected for possible motions of the five-membered spin label haptens as deduced from studies of molecular models.

Hapten	Allowed motion† (about the bond shown)	Spatial requirements for this motion (nm)† width	height	Calculated maximum value of hyperfine splitting constant for allowed motion	Observed maximum hyperfine splitting constant Enantiomer 1	Enantiomer 2
DNP-NH-CH$_2$ 	±40′	0.7	1.0	2.81	3 2	2.7
5-F-DNP-NH 	±20	0.6	0 9	3.1	3.0	2·63

* Chiral centre
† Calculated from atomic models.

of intrinsic allowed motions of these haptens (Table 1) assuming a rigid DNP ring leads to the determination of precise lateral dimensions These conclusions are summarised in Fig. 2.

Environment of hapten side chains

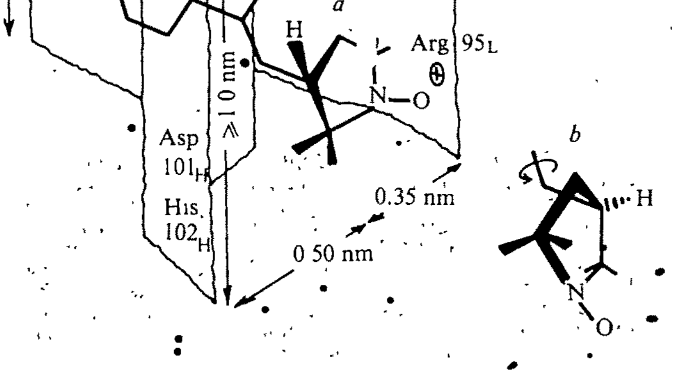
The polarity of the nitroxide radical's environment can affect the magnitude of the hyperfine splitting. The relatively large difference in A_{max} between the 'rigid' enantiomers of haptens V (3.2) and VI (3.0) (~0.2 mT, Table 1) is expected to arise from a polarity effect, that is the nitroxide of hapten V is probably close to a positive charge which results in a higher value of A_{max}. A positive charge 0.1–0.3 nm away from the NO and along its axis could account for the difference in the values of A_{max} (refs 12, 13) between the two haptens. The length and geometry of hapten V would then allow the positively charged residue to be placed as shown in Fig. 2 Reference to the predicted model (Fig 1) immediately suggests that this residue must be Arg 95$_L$, since hapten V is rigid and the NO lies along the axis of the DNP ring.

The use of ^{31}P magnetic resonance provides an additional and more powerful probe of electrostatic environment. Since the pK_a of a phosphonate grouping is extremely sensitive to neighbouring charges, changes in the ionisation state of ^{31}P groupings with pH can be relatively easily monitored by observation of the changes in ^{31}P chemical shifts The phosphonate grouping approximates to a hemisphere of radius 0.3 nm so that by using a series of side chains of different lengths it is possible to probe different areas of the combining site for positively and negatively charged groups.

The pK_a values of three TNP alkyl phosphonate haptens (methyl, ethyl and propyl) when either free in solution or when bound to the Fv have been measured from titrations of ^{31}P chemical shifts (at 129 MHz). Only the TNP propyl phosphonate has its pK_a significantly affected, being decreased by 0 8 unit (from 7.7 to 6.9) suggesting the proximity of positively charged group. Using molecular models the extended length of this hapten measured along the plane of the TNP ring is 1.2 nm. From the dimensions of the site obtained from the e s r. mapping studies this places the phosphonate grouping just beyond the entrance to the combining site and in a position to interact with the positively charged group detected by the e.s.r. studies. The pK_a mapping and the e.s.r mapping techniques are therefore complementary and reference to the model confirms that Arg 95$_L$ is the candidate for this positive charge in both cases. Lys 52$_H$ is another positively charged residue at the edge of the combining site as has been confirmed by affinity labelling with bromoacetyl DNP lysine[14]. However, consideration of the geometries of the Tnp phosphonate haptens and the affinity label indicate that the phosphonate ligands are considerably shorter and will not be able to interact with Lys 52$_H$. This further illustrates how different techniques can be used to probe different areas of the combining site.

The predicted model places one (His 102$_H$) out of the three histidines in the Fv fragment near the combining site His residues are particularly convenient to study by high resolution n.m.r. The pK_a values of the three histidine residues in the Fv fragment have found to be 5.9, 6.9 and 8 2 (ref 10). On hapten binding, only one His residue has its pK_a significantly affected, the pK_a being increased from 6.9 to 7.2–7.4 depending on the hapten side chains. The narrow linewidths of the His resonances of this histidine suggest that it is on the surface of the protein and readily accessible to solvent. Reference to the model suggests this can only be His 102$_H$ This means that the pK_a value of 5 9 can be assigned to His 97$_L$ since we have previously assigned a pK_a value of 8.2 to His 44$_L$ from the results of paramagnetic spin label difference spectroscopy[10] His 102$_H$

Fig. 2 The overall dimensions of the DNP binding site as determined by spin-label mapping. The two enantiomers of one of the five-membered spin-labelled haptens are shown Anomer a is rigidly immobilised but anomer b is free to undergo a restricted rotation as shown.



therefore provides a convenient marker of the edge of the site. His 102_H on the other hand cannot be the candidate for the positive charge discussed previously which is sensed by the spin labels and the phosphonate groups. This is because the pK_a of His 102_H is similarly affected by various DNP ligands regardless of their length or charge. Hence the change in His 102_H pK_a reflects local changes in its environment whereas Arg 95_L interacts specifically with charged groups of the hapten which have the appropriate length of the side chain.

The DNP binding site

The red shift in the optical absorbances of DNP ligands bound to protein 315 (ref. 5) and circular dichroism studies[16] indicate interaction between the DNP ring and a tryptophan residue. The complex formed between a DNP ligand and L-tryptophan was therefore used as a model for the DNP–Fv interaction in an n.m.r. analysis. The addition of varying amounts of DNP-aspartate into a 10 mM solution of L-tryptophan results in upfield chemical shifts of all the aromatic resonance. By appropriate titrations[16] estimates of the fully bound shifts of all the protons in the complex can be obtained. These are in the range 0.1–0.5 p.p.m. upfield. Replacing tryptophan by any of the other aromatic amino acids does not result in any observed shifts thus showing the specificity of the interaction between the DNP ligand and tryptophan. The upfield chemical shifts can be analysed in terms of ring current interactions[17–19] which relate the shift on a nearby nucleus to its position from the centre of its aromatic ring responsible for the shift. Since all the aromatic protons in this system are shifted upfield this immediately suggests that a stacking interaction is occurring. The structure of the DNP and TNP complex is then found by a computer search which tests all possible

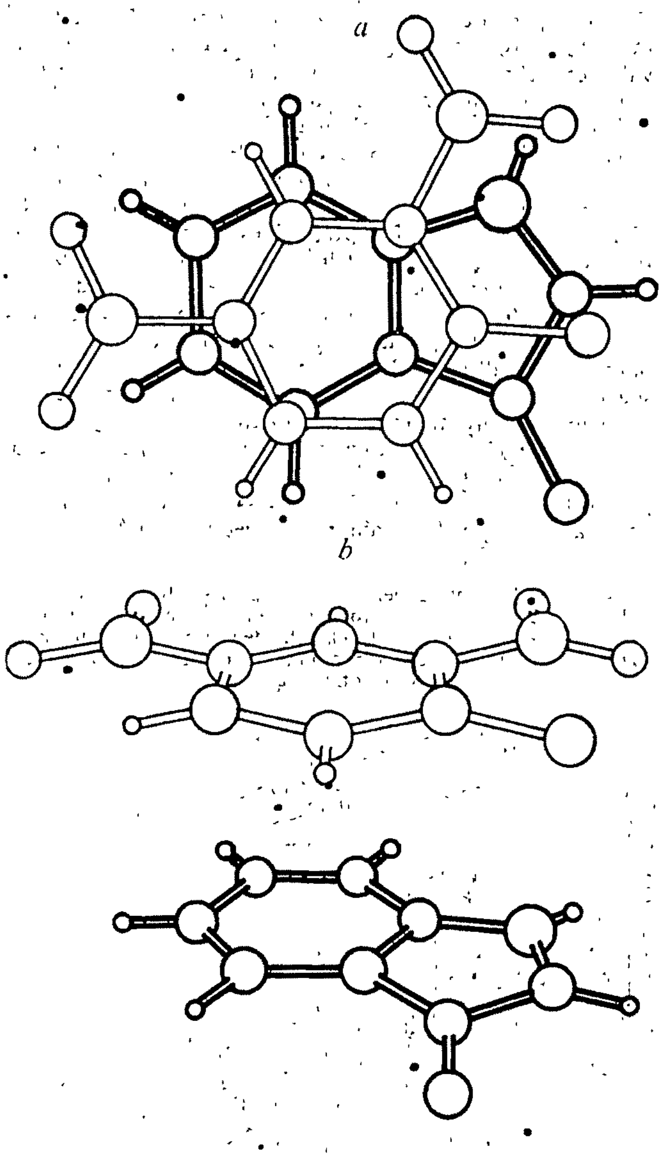


Fig. 3 The structure of the DNP-aspartate and tryptophan complex as deduced from ring current interactions. The two views are obtained from a computer search of the possible structures, with a separation between the two rings of 0.33 nm, which give calculated ring current shift ratios in best agreement with the experimental values. The shifts are calculated using the Johnson–Bovey equation modified to take into account the indole ring. The two views represent (*a*) a vertical view down the *z* axis and (*b*) the vertical view rotated 30° about the *z* axis and 60° about the *x* axis.

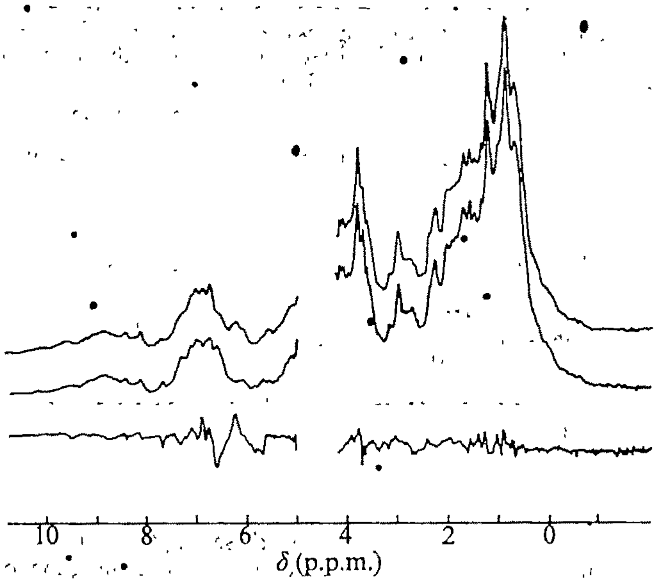


Fig. 4 The 270 MHz proton spectra of the Fv fragment alone and in the presence of Dnp aspartate and the difference spectrum between the two (enlarged × 4). Conditions were (Fv) = (Dnp-aspartate) = 1.15 mM, $pH = 6.9$, $T = 303$ K (0.15 M NaCl).

Table 2 Chemical shifts (δ_0) and the change in chemical shift ($\Delta\delta$) of resonances perturbed on binding of DNP-aspartate to the Fv from MOPC 315 at pH 6.9.

Hapten resonances	δ_0 (p.p.m.)	*$\Delta\delta$ (p.p.m.)	No. of protons
H_3	9.18	−1.68	1
H_5	8.36	−2.3	1
H_6	7.04	−1.31	1
—CH_2—	2.76	−0.1	
(Complex pattern with	2.91	−0.2	2
3 main peaks)	2.99	−0.26	
—CH—		not observable	
Protein resonances			
5.9 His C2	7.82	−0.06	1
—	7	broadens out	~2
5.9 His C4	6.95	−0.07	1
—	6.9	−0.19	1
—	6.62	0.08	1
—	6.37	0.295	2
	6.33	−0.175	<2
	6.16	−0.255	2
Peak decreases as hapten is added	7.2		~2
Peak increases as hapten is added	7.42	poss. 0 22	2

* Positive sign is downfield, negative sign is upfield shift change.

Measurements are made at 270 MHz, at $T = 303$ K and in the presence of 0.15 M NaCl. Shifts were measured from the sodium salt of 3-(trimethyl-silyl-propane) sulphonic acid, as an external standard.

geometries of a stacking arrangement of two rings. A closest distance of approach of the two rings of 0.33 nm is assumed since this is the distance found in a crystalline indole–TNP complex[20]. To avoid any problems from alterations in the strength of the ring current interactions from possible charge transfer and other effects the computer search uses the shift ratios. These ratios are then calculated for the protons in the complex in each geometry and these are tested against the experimental values. Two views of the structure of the complex as obtained from computer fitting are shown in Fig. 3. If this interaction occurs in the Fv–DNP-aspartate complex it would be expected that the n.m.r. spectrum of the complex should reveal upfield chemical shifts of similar magnitudes (that is 0.1–0.6 p.p.m.) for the DNP protons and of protons from the Trp residue in the Fv fragment.

The addition of varying amounts of DNP-aspartate to the Fv results in changes in the chemical shifts of the proton resonances at 270 MHz from both hapten and protein. The initial and final spectra are shown in Fig. 4, together with the difference spectrum between Fv and the 1:1 complex. If the histidine resonances are used as internal area markers only about 10 aromatic protons are perturbed on hapten binding. By means of difference spectroscopy the titration behaviour of all these resonances can be followed and the results are given in Table 2. On the protein, apart from the resonances assigned to a histidine (97$_L$, $pK_a = 5.9$) five protons have their resonances shifted upfield. The magnitude of these shifts are very similar to the DNP-induced ring current shifts of the tryptophan protons in the model complex. It seems reasonable to conclude that some, if not all, of the resonances in the protein which

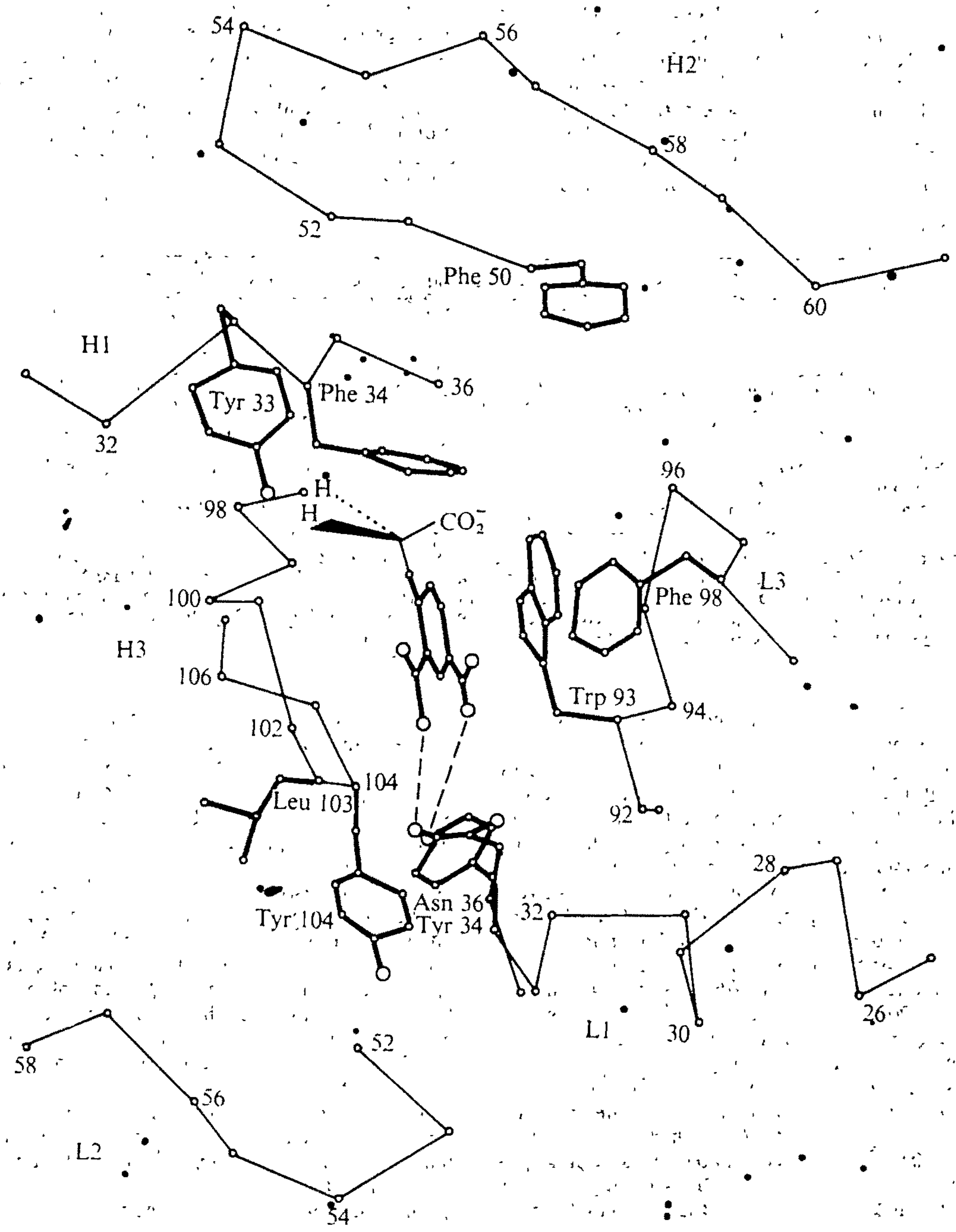


Fig. 5 The refined combining site of MOPC 315 based on the data in this paper. The positioning of Phe 34$_H$, Tyr 34$_L$, Trp 93$_L$ and Tyr 33$_H$ are obtained from ring current calculations. Tyr 104$_H$ can interact with Tyr 34$_L$. Some of the second layer of aromatic residues are shown. The orientations of the hapten is such that the two $-NO_2$ groups can form H bonds to Asn 36$_L$ and Tyr 34$_L$. Coordinates of this refined combining site are available on request.

are shifted upfield on addition of the hapten are from a tryptophan and that the DNP ring when bound in the Fv will interact with this tryptophan in a very similar manner to that shown in Fig. 3. Inspection of the proposed binding site in Fig. 1, immediately suggests that this tryptophan in the Fv is Trp 93$_L$.

The upfield shifts of the DNP protons when bound to the Fv are far larger than those in the DNP-aspartate–tryptophan complex. Analysis of these shifts reveals that it is necessary to invoke a minimum of two additional aromatic residues to explain the magnitude of these shifts One of these aromatic residues must be above and in almost van de Waal's contact with the H(5) and H(6) protons while a second aromatic residue is required to account for the shift on the H(3) proton. Reference to model suggests that these two residues are Phe 34$_H$ and Tyr 35$_L$. Hence the DNP ring is essentially enclosed in an 'aromatic box' consisting of Trp 93$_L$, Phe 34$_H$, and Tyr 34$_L$

The binding of DNP–glycine to the Fv provides the clue that now allows the orientation of the hapten in the site to be fixed. The side chain-CH_2-protons of this hapten experience a shift of 1 p.p.m. on binding to the Fv. To explain this shift it is necessary to introduce a fourth ring in the vicinity of the DNP situated about 0.3 nm above the CH_2 grouping of DNP–Gly. This can only be Tyr 33$_H$ which occupies similar position in the model. It is interesting that nitration of this tyrosine does not affect the binding constant of haptens to protein 315 suggesting that the interaction between Tyr 33$_H$ and the hapten is through the ring and not the hydroxyl. In contrast, in protein 603 an H bond is formed between Tyr 34$_H$ and the phosphate group of the phosphoryl choline hapten.

Consideration of geometry of DNP glycine then shows that the H(3) proton is on the opposite side of the molecule to the CH_2 Thus the H(3) proton must be over Tyr 34$_L$ while the H(5) and H(6) protons are in van de Waals' contact with Phe 34$_H$. Further refinement of the environment of Tyr 34$_L$ comes from the observation of an Overhauser effect[14] on the H(3) proton of the DNP ring: Irradiating the protein resonances in the Fv at 6.6 p.p.m. results in the reduction of the signal intensity of this H(3) proton. This implies that one or more protons on Tyr 34$_L$ (which have resonances at this position) are within 0.3 nm of the H(3) proton. Further the affected protons on Tyr34$_L$ must be influenced by another aromatic ring since the resonance at 6.6 p.p.m is shifted upfield by ~0 6 p.p.m. from the position expected for an unperturbed tyrosine residue. According to the predicted model this other aromatic ring can only be Tyr 104$_H$ but this necessitates repositioning this residue so as to be vicinal to Tyr 34$_L$. This is only possible if the position of Leu 103$_H$ is altered in the postulated model so as to point out of the site in which event Tyr 104$_H$ will point into it and can then interact with Tyr 34$_L$. This is also compatible with the lack of any large change in methyl resonance in Fv on hapten binding.

Other differences from the predicted model also arise from consideration of a 'second layer' of aromatic residues For instance the protons assigned to Trp 93$_L$ are themselves ring current shifted upfield from the normal expected positions in a protein. This suggests that Trp 93$_L$ points back into the site where the protons of the six-membered ring can be upfield-shifted by Phe 98$_L$ Finally the positioning of the hapten allows conclusions to be drawn about possible H-bonding schemes The two nitro groups on the hapten seem to be necessary for strong binding and suggest some H-binding interactions to amino acid side chains[3]. The only possible H-bonding groups which can interact with NO_2 groups on the DNP ring positioned as discussed above are Asn 36$_L$ and Tyr 34$_H$ Interestingly both these H bonds as well as the involvement of Trp 93$_L$ are on the light chain and may be the reason for a weak binding site on light chain[22] and the V_L dimer[23] for DNP ligands The refined model of the combining site modified according to the data in this paper is shown in Fig. 5 and illustrate the considerable agreement with the original model However, the n m.r. results allow very precise positioning of the contact residues and given the assumptions that aromatic rings are the only source of upfield shift interactions and that there is no motional averaging of these rings, the placing of the rings around the binding site is estimated to be accurate to ±0.05 nm.

Table 3 Comparison of combining site residues which interact with ligands*

Protein	Ligand	H1	H2		H3	
		31	50	59	99	
315	DNP	Ser Gly *TYR* Phe Trp† Asn	*PHE* Ile *LYS* Tyr		Asp Asn Asp His — — — — —	Leu
603	Phosphorylcholine	Asp — Phe *TYR* Met *GLU*	Ala Ser *ARG* Asn	… *GLU*	*ASN* Tyr Tyr Gly Ser Thr *TRP*	
New	Vitamin K	Asn — Asp Tyr Tyr Thr	*TYR* Val Phe Tyr		Asx *LEU ILE ALA* — — — — —	Gly
		L1			L3	
		32			92	
315	DNP	Ser Asn *TYR* Ala Asn			Leu *TRP* Phe *ARG* Asx His	
603	Phosphorylcholine	Lys Asx Phe Leu Ala			X X X X X	
New	Vitamin K	*GLY ASN* His Val Ser			Ser *TYR* Asp Arg *SER LEU*	

* Interacting residues (italic) are according to refs 1 and 2 for New and 603 respectively and from this work for protein 315. When no residues are marked the possibility of interacting with peptide chain backbone is not excluded. H1, H2, H3, L1 and L2 are hypervariable regions of H and L chains. X, unknown residue due to incomplete sequence The numbering of sequences is according to protein 315 with homology maximised. We thank Professor R. J. Poljak for the revised sequence of protein New residues 31–35 before publication

† Changed from lysine after partial resequencing (D. Givol, personal communication).

Several interesting points emerge from the comparison of the combining site of protein 315 with those of protein New and 603. In Fab' New-vitamin K_1OH complex, the naphthoquinone ring of vitamin K_1OH is in close contact with Tyr 90$_L$[1] (Tyr 93$_L$ in Table 3) Sequence homology shows that Trp 93$_L$ in protein 315 which interacts with the DNP is homologous to Tyr 90$_L$ in New, and therefore this provides an interesting example for a change in specificity on replacing one residue in the site. Note that protein 315 also binds menadione in lower affinity than DNP (ref 24), but protein New does not bind DNP ligands. Charge interactions play a dominant part in the binding of protein 603 to the hapten phosphoryl choline[2] On the other hand, another model building study on rabbit anti-type III polysaccharide (BS-5) suggest clustering of the hydroxyamino acids Tyr, Ser and Thr and also Asn in the combining site which can form H bonds with hydroxyls of the sugar ligand[25]. It is clearly seen that some pattern of changes in the antibody binding site can be deduced from such comparative studies as illustrated in Table 3.

The magnetic resonance study of interaction between haptens and antibody provides a functional sense to the structure predicted by model building. It is possible to assign interactions to residues in the site and also to determine their correct orientation with respect to the hapten. The positioning of Trp 93_L, Phe 94_H, Tyr 34_L, Asn 36_L, Arg 95_L and other residues allows understanding of the specificity of binding DNP ligands to protein 315 and indicates that this approach when applied to other antibodies will provide information about the structural basis of antibody specificity.

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letters to nature

Acceleration of charged particles to extremely high energies in pulsar far fields

SINCE the inception[1] of the pulsar magnetosphere, it has been realised that charged particles, streaming away from the neutron star beyond the light cylinder, would attain high energies. Goldreich and Julian[1] calculated the potential drop Φ_M across the open field lines at the polar cap of the star, noting that this was the order of the maximum energy that such particles could attain. Φ_M is of order 10^{16} V for the Crab pulsar. They estimated (their equation (34)), however, that the energy actually reached would be very much less than this. I show here that in fact all escaping particles will reach energies of order Φ_M.

We assume that the electromagnetic field is given adequately by the force-free approximation[2,3], in which particle mass is neglected, and then calculate the motion of charges in this field to first order in the mass. This leads to an estimate of the region of adequacy of the approximation.

If the mass is neglected altogether, the motion is simply

$$\mathbf{v} = \mathbf{U}_E + v_{\parallel}\mathbf{B}/B \tag{1}$$

where $v_{\parallel}$, the component along the magnetic field $\mathbf{B}$, is unrestricted. In force-free theory[4], the electric field, E, is given by

$$\mathbf{E} = -\mathbf{v}_R \times \mathbf{B} \tag{2}$$

where $\mathbf{v}_R$ is the co-rotation velocity $\Omega \times \mathbf{r}$, Ω being the angular velocity of the star. Thus the electric drift $\mathbf{U}_E$ is

$$\mathbf{U}_E = \mathbf{E}\times\mathbf{B}/B^2 = \mathbf{v}_R - (\mathbf{v}_R\cdot\mathbf{B})\mathbf{B}/B^2 \tag{3}$$

At large radial distances, $\mathbf{U}_E$ becomes radial[5-7], and equal to c (within the force-free approximation). In the general, non-axisymmetric case[7], the far field is dominated by an electromagnetic wave (of frequency Ω), for which $E/B = c$, so this result is clear.

It is essential within the approximation that $\mathbf{U}_E$ should not exceed c. The Lorentz factor γ_E corresponding to $\mathbf{U}_E$ follows from (3):

$$\gamma_E^2 = B^2/\mathbf{B}\cdot\widetilde{\mathbf{B}} \tag{4}$$

where

$$\widetilde{\mathbf{B}} = (1 - v_R^2/c^2)\mathbf{B}_p + \mathbf{B}_t \tag{5}$$

The derived field $\widetilde{\mathbf{B}}$ is of fundamental importance in force-free theory[2,3]. We require therefore for the validity of the approximation, that

$$\mathbf{B}\cdot\widetilde{\mathbf{B}} \geqslant 0 \tag{6}$$

It follows from the universal spiral structure of the far field[7] that whereas B^2 decreases as r^{-2}, $\mathbf{B}\cdot\mathbf{B}$ decreases as r^{-4}. There is a great degree of arbitrariness at present in the directional dependences of the quantities, but we assume that there exists a class of solution for which (6) is valid. At large r therefore,

$$\gamma_E \simeq F r/r_l \tag{7}$$

where r_l is the light radius c/Ω, and F is some function of direction.

To zero order in the mass therefore, the particles gain energy in proportion to their distances from the star. To find out when inertial forces will curb this process, we must calculate the first order 'guiding centre drift'. This depends partly on $v_{\parallel}$, calculation of which in general[8] necessitates integration of an equation for $dv_{\parallel}/dt$. Here, this is unnecessary because of the existence of an exact integral of the motion due to Endean[9],

$$\mathcal{E} = \gamma(1 - \mathbf{v}_R\cdot\mathbf{v}/c^2) \tag{8}$$

This arises from the requirement that the whole system be stationary in the co-rotating reference frame. When $\mathbf{v}$ is inserted from (1) into (8), there results a quadratic equation for $v_{\parallel}$ whose coefficients depend on $\mathbf{B}$ and $\mathcal{E}$. For values of r sufficiently large that (7) is adequate, the physical root of this equation, and the associated value of γ are approximately followed by equation (9):

$$v_{\parallel}/c \simeq -(\sigma\, \mathscr{E}/(\sin\theta F))\,(r_l/r)^2, \qquad \gamma \simeq \gamma_E, \tag{9}$$

where θ is the polar angle, and σ is the sign of B_t.

The calculation of the drift is (apart from straightforward relativistic extensions) standard[10]. We assume that any gyromotion is lost by synchrotron radiation near the light cylinder. Drifts involving $v_{\parallel}$ turn out to be negligible because of its r^{-2} dependence. (Thus the motion is eventually independent of initial conditions which appear through $\mathscr{E}$.) The dominant drift is

$$v_{\perp} = \frac{m}{q}\left\{\mathbf{B}\times \mathrm{d}(\gamma \mathbf{U}_E)/\mathrm{d}t\right\}\Big/B^2 \tag{10}$$

evaluated along the zero order trajectory. The corresponding corrected γ reduces eventually to

$$\gamma \simeq F(r/r_l) + \delta G(r/r_l)^2 \tag{11}$$

where G is a second function of direction obtainable from a given force-free model, and δ is the small number

$$\delta = \Omega/\omega_{BL} \tag{12}$$

where ω_{BL} is a measure of the non-relativistic gyro-frequency at the light radius. For the Crab pulsar δ is of order 10^{-11} for electrons and 10^{-8} for protons.

The drift approximation will fail when the two terms in (11) are comparable. We can only speculate on what occurs beyond this but it seems likely that gyro-motion will appear[1], and that there will be no further energisation. We assume that this is the case. In a given direction there will be a maximum value γ_M, and corresponding energy K_M, at a distance r_M where

$$\begin{aligned} r_M &= R\, r_l/\delta, \\ K_M/mc^2 &= \gamma_M = \Gamma/\delta, \end{aligned} \tag{13}$$

where, roughly, $R \approx F/|G|$ and $\Gamma \approx 2F^2/|G|$. We assume that the field falls as r^{-3} from the star to the light radius, thence as r^{-1}. If the stellar radius and field strength are $R_s \times 10\ K_M$, and $B_s \times 10^8$ T, respectively, then

$$\begin{aligned} r_M/R = e\Omega R_s^3 B_s/mc^2 &= 6\times10^{-3}\ \Omega R_s^3 B_s \text{ pc (electrons)} \\ &= 3\times10^{-6}\ \Omega R_s^3 B_s \text{ pc (protons)} \\ K_M/\Gamma = e\Omega^2 R_s^3 B_s/c &= 4\times10^{11}\ \Omega^2 R_s^3 B_s \text{ V} \end{aligned} \tag{14}$$

K_M has the same order as the Φ_M of Goldreich and Julian[1], and is about 2×10^{16} V for the Crab pulsar. The field at r_M is such that the non-relativistic gyro-frequency is comparable with Ω. This is of course expected where the drift approximation breaks down It is much larger than any possible interstellar field for protons, but for slower pulsar periods of order 1 s, it is of order 10^{-10} T for electrons. Electrons therefore, may be lost from the system before attaining their maximum energy. The distance r_M would seem to be well within the surrounding nebula of any pulsar.

A rough estimate of the flux of energy from the system carried by the particles yields a figure comparable to the dipole radiation rate. Again then, it is clear that the approximations break down at r_M, and that a self-consistent approach must replace the force-free approximation at such distances.

Finally, the loss of energy ΔK due to radiation reaction during the motion is negligible. This is basically because particles are travelling at the speed of light in straight lines. Using standard formulae[11] it is readily shown that the ratio $\Delta K/K_M$ is of order Ωt_r, where t_r is the classical radiation time scale 10^{-23} s (electrons). Thus ΔK is completely negligible.

Further details of the process, and the breakdown of the force-free approximation will appear elsewhere.

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Redshift effect on the timescale of quasar radio variability

It has been suggested (ref. 1 and McCrea (I.A.U. Symposium No. 63, 1972)) that the radio or optical variability of quasars should be related to their redshifts. If the latter originate from the Doppler effect, or are gravitational, the observed fluctuation rate of the radio or optical emission should also be redshifted, that is, slowed down, in proportion to $1+z$, where z is the usual redshift factor. Medd *et al.*, measuring quasar flux densities with the Algonquin telescope, attempted to detect this effect by plotting the frequency of maxima occurrence in the flux-density curves against z but obtained an inconclusive result. Scargle[2] also analysed the Algonquin observations, using the half-width of the autocorrelation function of the flux-density curve as a measure of fluctuation rate. Again, the results show considerable dispersion, but Scargle argues that regression analysis supports the expected relationship. We present here a preliminary version of an analysis based on the power spectrum of the flux-density curves, which establishes the expected result.

Our analysis was restricted to the λ2.8 cm measurements (ref. 1 and B. H. Andrew and G. A. Harvey, personal communication) of the 33 identified quasars in the Algonquin sample for which redshifts were available. The data covered periods of 74–110 months between 1966.5 and 1975.7, a month being defined as 1/12 yr. The record lengths are variable, therefore; moreover, they contain gaps and the observations are not equally spaced in time.

The data were interpolated to produce equally-spaced samples at 1-month intervals by a weighted linear interpolation (convolution); the convolving function was triangular, with a base of 6 months. (This has a significant filtering effect on the power spectrum which was subsequently corrected.) Where the gaps were too wide to use this function, the data were interpolated linearly The d.c. component of the record, and any linear trend, were removed by fitting a straight line in a least-mean-squares sense A cosine taper was applied to 10% of the record length at each end, and all records were converted to a uniform length of 128 months by adding zeros at the end. The resulting records are 128 samples long, appropriate for applying the Fast Fourier Transform. The transformation results in spectra which are tabulated at intervals of 1/128 = 0.00781 cycle per month. The spectral amplitudes were then squared to arrive at the power spectra of the flux-density curves.

A typical spectrum is shown in Fig 1*a*. At frequencies < 0.01 cycle per month, a finite record length coupled with the absence of d.c. power biasses the first three points downwards. At high frequencies the spectrum consists only of noise, representing the original measurement errors in the flux densities Between these extremes is a regime where ln$P$ falls approximately linearly with frequency, shown more clearly in Fig. 1*b* All 33 quasars have a similar form of spectrum, though noise makes the similarity less clear-cut for those which vary weakly in comparison with the flux measurement errors. Attention was therefore restricted to those 13 quasars for which the s.d. of the time-series record exceeded that of the quoted measurement errors[1] by a factor of 1.7, which corresponds to a convenient break in the distribution of s.d. ratios.

If the variability spectrum of a given quasar could be measured for a redshift $z = 0$ then at a finite redshift z, the effect on the spectrum would be to steepen the slope $\mathrm{d}(\ln P)/\mathrm{d}f$ by the factor $1+z$. A plot of slope against $1+z$ for different quasars might also be expected to show this relationship, provided the dispersion in variability rate from one quasar to another is not so strong as to obscure it.

For each quasar, the slope was measured by computing the average values P_1 and P_2 of P over the frequency ranges 0.0117–0.0508, and 0.0508–0.0898 cycles per month, and forming the quantity $(\ln P_1 - \ln P_2)/0.0391$. The averaging ranges were chosen as a compromise between a wide range, which would give a good estimate of the slope, and the necessity to restrict the measurement to that part of the spectrum having a high signal-to-noise ratio. The results are plotted in Fig. 2. There is clearly evidence for a correlation in the expected sense.

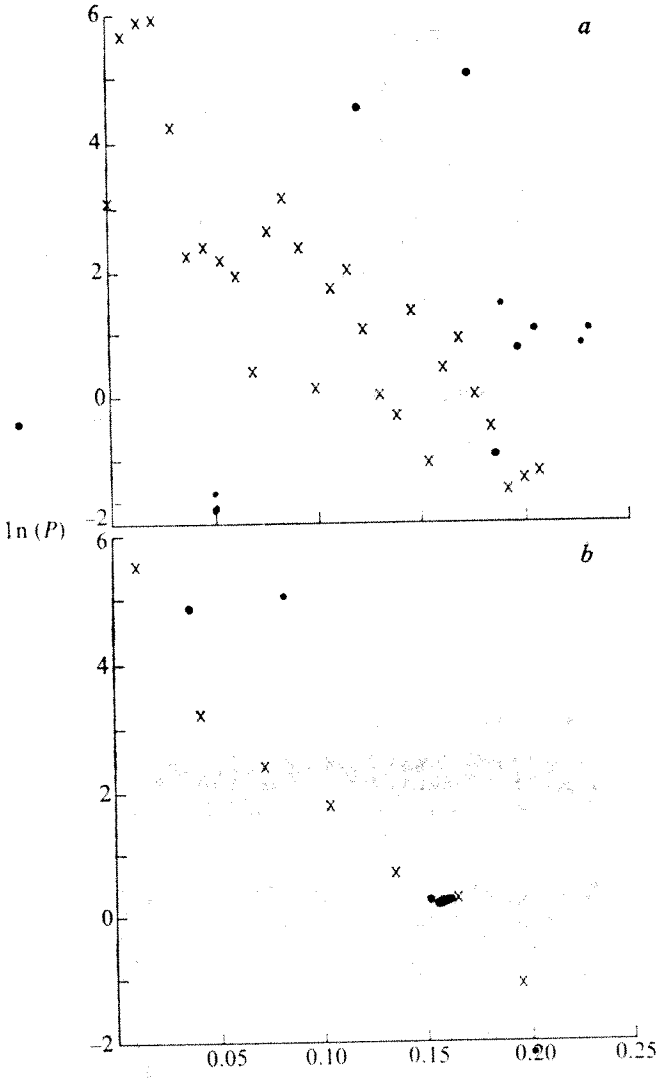

Fig. 1 Power spectra of the radio flux variations of CTA 26, corrected for the filtering effects of the convolving function. The ordinates are the natural logarithms of the power spectral density P, in units of Jy^2 (cycles per month)$^{-1}$. a, raw spectrum at a resolution of 0.0078 cycles per month. b, spectrum smoothed by averaging in groups of four points.

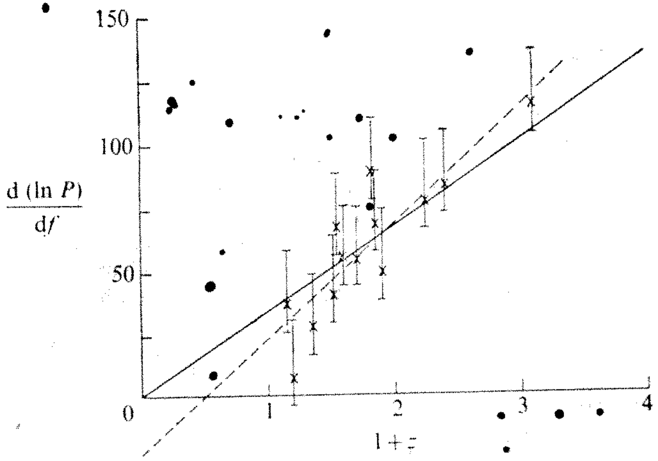

Fig. 2 Plot of $\mathrm{d}(\ln P)/\mathrm{d}f$ against $(1+z)$. The full line is a regression of y on x which is constrained to pass through the origin. The dashed line is not so constrained. The 1σ confidence bars are derived from the χ^2 distribution of the spectral estimates.

Assuming that the time-series are random and not deterministic, and that they are noise-free, spectral estimates will follow a χ^2 distribution. Since the data were selected to have a high signal-to-noise ratio, this will be very nearly correct. Confidence limits of 1σ have been computed (Fig. 2). The assumption of randomness is crudely verified *post facto* as the χ^2 estimates seem to be consistent with the observed scatter in the spectra.

Figure 2 shows a correlation in the expected sense between the spectral slope and the redshift, and the value of the correlation coefficient is computed to be 0.88. It is, however, difficult to set a proper confidence limit on this result, because the usual tests are based on a normal distribution of errors in the data, which is not the case here. Nevertheless, we have assumed normality, to set a rough confidence limit on the result, an assumption which finds crude justification in the central limit theorem[3]. The correlation coefficient is then significant at a level much lower than 1%.

Although the correlation is significant, the expected proportionality between spectral slope and $(1+z)$ should cause the regression line to pass close to the origin. In fact, its intercept on the y-axis is -26.6, but the significance of this departure is only 7% (again assuming normality) so the fact that the regression line does not pass through the origin is not very significant. The regression of y on x with the line constrained to go through the origin is shown in Fig. 2.

Our establishment of the expected correlation between the observed rate of radio variability and redshift implies that whatever causes the redshift of the optical spectral lines must also slow down the observed variability rate. Because the unshifted variability rate is not known *a priori* (unlike the frequency of optical spectral lines), we cannot establish that the radio variability is subject to the same value of $(1+z)$. Our data would be consistent, for instance, with an interpretation in which the radio emission were subject to exactly half the optical value of $(1+z)$. If the redshift is supposed to have a Doppler (or a gravitational) origin, our data require that the optical and radio emission arise in regions with equal or related recessional velocities (or gravitational potentials).

An interesting inference from Fig. 2 is that the intrinsic spread of quasar variability rate (at a given redshift) is low, at most of the order of 3, and presumably lower if the effects of the χ^2 variance were reduced, by accumulating longer records. This suggests that variability rate may be a useful parameter for cosmological investigations, assuming the redshift is cosmological, and could be used, for instance, to measure redshift as a function of cosmological epoch.

The exponential power-spectrum of quasar variability can be useful in considering the astrophysics of these objects. If the form of the spectrum is $P = Ae^{-kf}$, then the mean value of can be deduced from Fig. 2 using the value of the ordinate of the full regression line for $z = 0$. We obtain $k = 33.3$ (cycles per month)$^{-1}$, implying that most of the variations take place at frequencies $< k^{-1} = 0.03$ cycle per month.

Further work clearly depends on acquiring more data, and on minimising the effects of errors on the present data. The number of points in Fig. 2 could be more than doubled if the 20 weakly-variable quasars in our original sample could be used.

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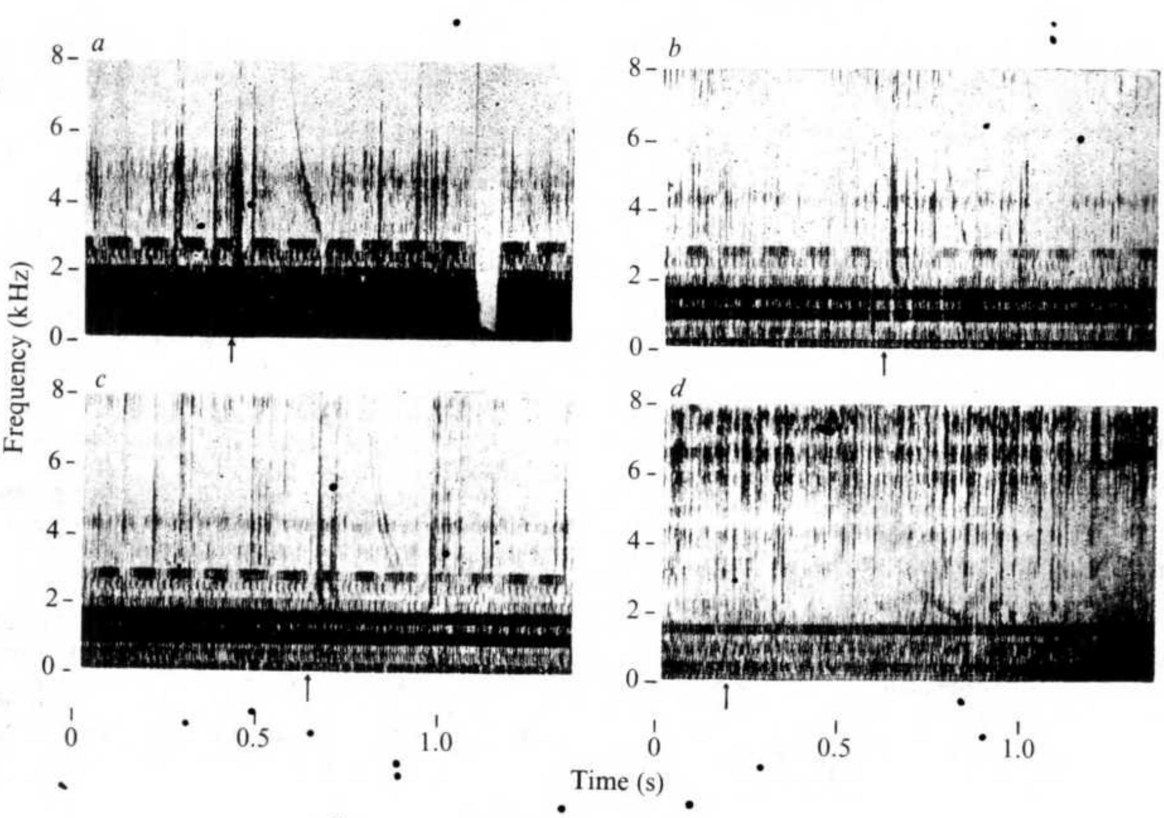
Whistlers recorded at Varanasi

THE study of low-latitude whistlers dates back to the pioneering work of Japanese scientists. The whistlers were first recorded at Toyokawa (geomag. lat., 24.5°N)[1], and later efforts were made to record them at lower latitudes. The first successful records of whistlers in India were obtained at Gulmarg (geomag. lat., 24°10′N) by Somayajulu *et al.*[2]. Recording was later carried out at Nainital (geomag. lat., 19°1′N) and whistlers with comparatively lower dispersion and reduced rate of occurrence were reported[3–5]. Unsuccessful efforts were also made to record whistlers at Varanasi (geomag. lat., 15°6′N). Analysis of the low latitude whistler records by Rao *et al.*[6] showed that the low-latitude whistler cut-off lies around 15° latitude, and apparently provided the reason for the non-recording of whistlers at Varanasi. Using an improved system, we have succeeded in recording whistler activity at Varanasi, and we describe here the categories of sonograms recorded in April, 1976.

It is well known that the global thunderstrom activity is at a maximum at the temperate latitude and decreases with increasing latitudes[7]. The parameters or mechanisms controlling the guidance of thunderstorm-generated low-frequency waves are less effective at low latitude, however, which seems to reduce the occurrence of whistler activity. The combination of these two controlling factors maximises the rate of occurrence around 50° geomagnetic latitude and results into a low-latitude whistler rate cut-off. It is obvious that the sporadic redistribution of thunderstrom activity and so-called enhancement factor controlling the guidance of whistlers at times changes the latitude of maximum occurrence rate of whistlers and shifts the low-latitude cutoff to still lower latitudes.

The interpretation of such a low-latitude cutoff seemed artificial and efforts to record whistlers at Varanasi continued, using an improved antenna system and preamplifier. The recorded sonograms (Fig. 1) show much noise with very distinct causative atmospherics. The whistler activity at Varanasi showed highest occurrence rate during the months of February–April, 1976, and generally occurred in the post-midnight hours. They can be classified into four different categories. Figure 1*a* shows a single trace short whistler with distinct causative atmospherics; the dispersion is 12 s$^{1/2}$. Figure 1*b* shows a feeble trace of banded whistler with almost similar dispersion features. The bands at lower frequencies are very clear, but are not distinct at higher frequencies. Figure 1*c* depicts two causative atmospherics

Fig. 1 Sonogram of whistlers recorded at Varanasi. *a*, 0026, 7 April 1976, short whistler; *b*, 0106, 4 April 1976, banded whistler; *c*, 0039, 7 April 1976, twin whistler; *d*, 2242, 4 April 1976, whistler with high dispersion. A model UHER 4400 Report stereo tape recorder provided the continuously recorded sonograms.



and gives an initial impression of multiflash, but dispersion analysis showed this to be a short twin whistler. Multiflash whistlers have not yet been recorded at this station. The dispersion feature is almost similar to that shown in Figs 1*a* and *b*. A curious but frequent trace is shown in Fig. 1*d*, where only a part of the trace in the low-frequency region (2–4 kHz) is seen and the high-frequency part of the trace does not appear. The extrapolation of the trace and the location of causative atmospherics shows a comparatively large dispersion of 25 $s^{1/2}$. The non-appearance of high-frequency part in this sonogram associated with this higher dispersion is capable of revealing certain additional features of low-latitude propagation path. To our knowledge such records have not been reported either at high- or low-latitude stations. The non-appearance of the trace at frequencies higher than 4 kHz is related, either to the enhanced absorption or to poor guidance of whistlers. Examination of the sonograms shows that such traces are often seen before or after the formation of banded whistlers (Fig 1*b* and *d*). It therefore seems less probable that the enhanced average absorption of the propagation path is a likely cause of absence of high-frequency trace of the sonogram. At the first glance the absence of high-frequency trace can be visualised as a particular case of banded whistlers which are believed to be some manifestation of shape, size and movement of ionospheric irregularities[8]. A detailed dispersion analysis of these sonograms is now being carried out to study various ionospheric and magnetospheric features.

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Argon degassing models of Mars

ON July 20, 1976 Viking I lander made a soft landing on the martian surface. Atmospheric pressure recorded on the surface by the lander was 7.7 mbar and main constituents of the atmosphere were 95% CO_2, 2–3% N_2, 1–2% Ar and 0.3–0.4% O_2 (ref. 1). The (^{40}Ar/^{36}Ar) isotopic ratio was 2,750 ± 500 as measured by a mass spectrometer[1]. This high isotopic ratio suggests an unique history for the evolution of the martian atmosphere. The possible mechanisms of argon degassing models of Mars are discussed here.

Since the decay of radioactive ^{40}K produces ^{40}Ar, the time variation of (^{40}Ar/^{36}Ar) isotopic ratio in a closed system can be determined by two initial ratios, (^{40}Ar/^{36}Ar)$_0$ and (^{36}Ar/K)$_0$. For the present case (^{40}Ar/^{36}Ar)$_0$ at the time of the planetary formation is considered to be very small ($\sim 10^{-4}$) from the theory of nucleosynthesis[2]. Hence the initial (^{36}Ar/K) ratio uniquely determines the later (^{40}Ar/^{36}Ar) ratio within the planet. The time variation of the isotopic ratio (^{40}Ar/^{36}Ar) as a function of the initial (^{36}Ar/K)$_0$ ratio is shown in Fig. 1. Observed (^{40}Ar/^{36}Ar) ratios in the terrestrial and the martian atmosphere are indicated by the arrows in the figure.

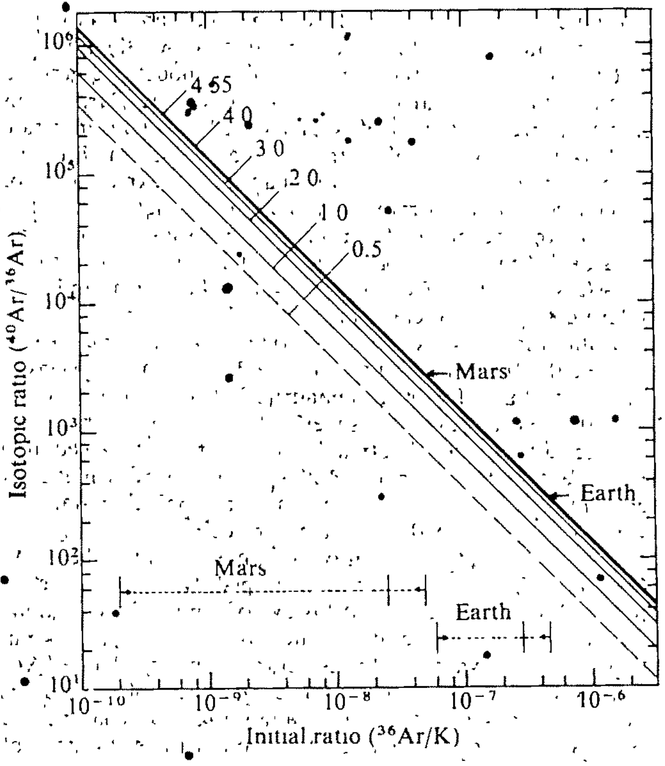

Fig. 1 Time variation of (^{40}Ar/^{36}Ar) isotopic ratio in a closed system as a function of initial (^{36}Ar/K) ratio. Two arrows indicate the isotopic ratios in terrestrial and martian atmospheres. Estimated ranges of the initial (^{36}Ar/K) ratio for Earth and Mars are shown in the lower part.

The present isotopic ratio (^{40}Ar/^{36}Ar) in the atmosphere depends on the degassing history of Ar from the planetary interior, so these observed atmospheric values can not determine the initial (^{36}Ar/K)$_0$ ratio. The possible range of the ratio, however, can be inferred from the observed isotopic ratio. If we assume the atmospheric Ar is degassed fraction from the planetary interior, the (^{40}Ar/^{36}Ar) ratio in the interior should be greater than the atmospheric value. This gives a maximum estimate for the initial (^{36}Ar/K)$_0$ ratio. For the Earth this value is about 4.5×10^{-7}. For the minimum estimate of (^{36}Ar/K)$_0$, we can assume ^{36}Ar has completely degassed to the atmosphere. This gives a value of 6×10^{-8} for the Earth with a chondritic mantle. The same argument suggests a possible range, $2 \times 10^{-10} <$ (^{36}Ar/K)$_0$ $< 4.5 \times 10^{-8}$, for Mars. These estimated possible ranges are shown in the lower part of Fig. 1.

Assumptions on the degassing model of Ar are needed to narrow the range. Turekian[3], assuming a first-order rate process for Ar degassing, obtained the degassing rate constant from the abundance of ^{40}Ar in the terrestrial atmosphere. Using his result and the atmospheric ^{36}Ar abundance, the initial (^{36}Ar/K)$_0$ ratio is estimated to be about 3×10^{-7}. Although he assumed a chondritic Earth (K content 880 p.p.m.), the initial ratio is almost independent of the assumption of K content in the Earth. Ozima[4] concluded initial catastrophic and later continuous Ar degassing model from the suggested high (^{40}Ar/^{36}Ar) ratio (more than 2,000) in the present mantle. This model requires a smaller (^{36}Ar/K) ratio than the continuous degassing model. The continuous degassing model for the martian atmosphere gives a value of 3×10^{-8} for the initial (^{36}Ar/K) ratio

This analysis of the Ar degassing model suggests a smaller (^{36}Ar/K)$_0$ ratio for Mars than for the Earth. This implies a higher content of K and/or a lower concentration of ^{36}Ar in the whole of Mars than in the Earth. Physical adsorption is the only known quantitative explanation of planetary primordial rare gas in chondritic meteorites[5] The size of the adsorbed fraction is very sensitive to temperature condition and increases as temperature decreases. As Mars is further from the Sun than the Earth, and considering the pre-planetary history of the solar nebula[6], it is probable that Mars is at least as rich in original volatiles as the Earth It seems likely that the K content of Mars is similar to that of normal chondrites[7] (900 p p.m.). Even if we assume that the atmospheric Ar on Mars consists of very recent degassed fraction from the interior, the required K content of Mars is about 1,200 p.p m An even higher K content is necessary to explain the apparent stational volcanic activity on the martian surface[8].

To overcome this difficulty, we must assume that ^{36}Ar (and probably other volatiles) escaped from the martian region during the geological history of Mars This escaping mechanism should operate in the early stage of the evolution of Mars, where (^{36}Ar) is more abundant than (^{40}Ar) The explanation of the chemical composition of the planets by the condensation theory[6], requires that the solar nebula escapes from the inner planetary region at the early stage of the solar evolution This process may contribute to remove the initially degassed volatiles from Mars Although the above discussion is based on the atmospheric content of Ar, the degassing history of volatiles closely related to the differentiation process in the planet, which can be inferred from petrological investigations of surface rocks as studied for the Moon[9]

If such investigation reveals an early large-scale differentiation of Mars, the above conclusion would be confirmed I thank Professor M Ozima for valuable discussions and encouragement

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Conductivity dispersion in single-crystal β-alumina electrolyte

WE report data which demonstrate the existence of a conductivity dispersion in single-crystal β-alumina and, we believe, for the first time, a distribution of conductivity relaxation times in any single-crystal ionic conductor A cylindrical boule of high quality Na β-alumina grown at Union Carbide was supplied to us by Roditi International Corporation. Figure 1 shows the frequency-dependent conductivity of this crystal in the range 10^2–10^7 Hz, measured at various subambient temperatures (for details of the apparatus see refs 1 and 2) At normal temperatures, electrode effects produce an apparent dispersion in conductivity At these lower temperatures, however, the dispersion seems to be an intrinsic property of the bulk material since it starts at frequencies which are related to the level of the d c. conductivity ($f \approx \sigma/2\pi\epsilon$)[1,2] as indicated by arrows in (Fig 1) This behaviour closely resembles the dielectric behaviour which has been studied in silicate and other glasses[3,4]. The dispersions in conductivity cannot be accounted for in terms of the conventional (Debye) theories of dielectric loss, indeed, the departures from Debye behaviour are probably more marked in single-crystal β-alumina than in many vitreous electrolytes

This result can be interpreted in terms of a broad distribution of conductivity relaxation times, which could plausibly be related to the random distribution of sodium ions over distinct crystallographic sites within the conduction planes (as revealed for example by recent X-ray and neutron diffraction studies)[5,6] These studies have shown that at ≲90 K, sodium ions are localised at three distinct sites, but with increasing temperature there is increasing cation mobility, and by 620 K the disorder resembles that of a two-dimensional fluid. The details of this picture have been confirmed by NMR spectroscopy[7], but of special relevance to this discussion, it should be noted that the existence of a 'wide distribution in ion jump frequencies' was postulated[5] to account for certain aspects of the data The conductance dispersion which we have observed would then follow directly from a model involving sequential hopping of ions over the different crystallographic sites

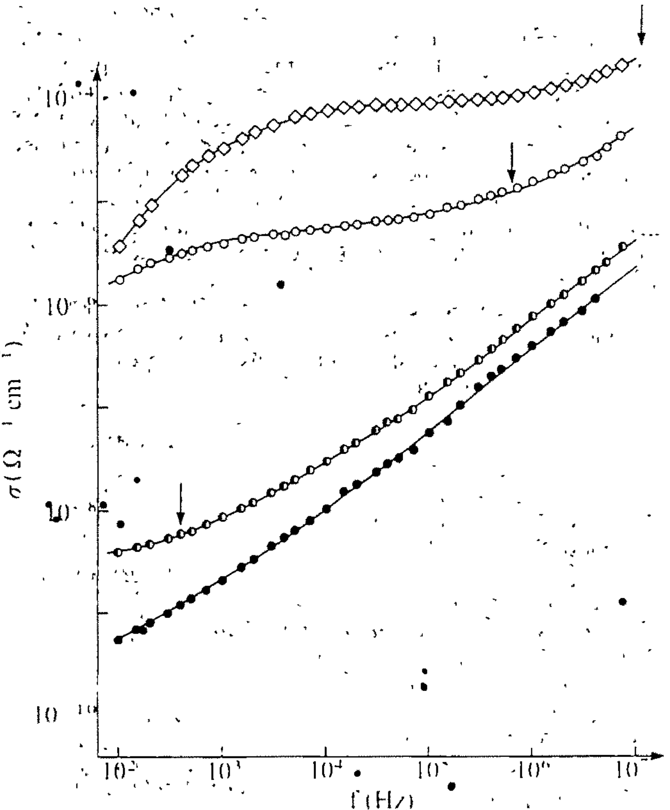

Fig. 1 Frequency-dependent conductivity of single-crystal β-alumina at 77 K (●), 87 K ( ), 113 K ( ) and 143 K (◇)

It is also possible, however, to place a different interpretation on these data In Fig 1, it is apparent that at low temperatures, the high-frequency or a c. conductivities fit a 'universal law' which has been described by Jonscher[8–10]. In the region of conductance dispersion, the a c conductance $\sigma(\omega)$ is given by:

$$\sigma(\omega) = A\omega^n \tag{1}$$

where n = 0·78 at 87 K, and is somewhat insensitive to changes in temperature. This 'law' is obeyed by a wide range of materials, and an interpretation has been given by Jonscher in terms of a 'screened' charge model where the ratio of energy stored to energy lost per cycle is independent of frequency On the basis of this theory the conductivity dispersion in β-alumina need not be related to a microscopic distribution of ionic jump frequencies, but could be understood in terms of an interionic 'drag effect' which gradually relaxes with increase in frequency. (It is tempting to relate this approach to a previous treatment of dielectric loss in vitreous materials which invoked the Debye–Falkenhagen effect[11].)

It is difficult to say that either of these explanations is 'right' and the other 'wrong'. Thus, according to the Jonscher model, highly 'lossy' electrolytes, such as β-alumina, need a high concentration of mobile ions to give effective charge-screening, but these are precisely the electrolytes which have a strong tendency to cation disorder and hence are likely to exhibit a wide spread in ionic jump frequencies[12]. The main point which emerges from the above discussion is a need for further detailed comparisons of structural, spectroscopic and electrical data, and hence the need for work with crystalline rather than vitreous systems. β-alumina is in many ways a model system, since measurements can be made over a wide range of d.c. conductivities, and extensive cation substitution is possible[13,14].

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The behaviour of long molecules diffusing in solid polyethylene

SEMI-CRYSTALLINE polymers, according to the current two-phase model, consist of an impermeable, denser crystalline phase (the lamellae) and a permeable, less dense amorphous phase (the interlamellar regions)[1]. All permeation and transport properties occur predominantly in the latter, and are a function of the overall amorphous fraction within the bulk and the geometry of the crystallites embedded in the amorphous phase. For linear polyethylene cooled at various rates from the melt, the diffusion coefficient D of gaseous diffusants varies[2,3] according to

$$D \propto (1 - x), \qquad (1)$$

where x is the corresponding crystalline volume fraction, and $(1 - x)$ the amorphicity. The diffusional behaviour of long chain diffusants, however, may be very different. For example, it has been suggested[1] that the transport of long chain molecules through the disordered interlamellar regions of polythene will be greatly influenced by the presence of interlamellar tie-molecules and entanglements. These are relatively immobile features in the disordered region which can obstruct the movement of long chain molecules, but not that of smaller (for example, gaseous) diffusants if their size is less than the separation between these features. An extension of de Gennes' ideas on reptation[3] gives a semiquantitative relation for the diffusion coefficient of long molecules in polyethylene: long molecules should diffuse faster in slowly cooled than in quenched polyethylene, in spite of the higher disordered fraction in the latter and in marked contrast to the behaviour of gaseous or light vapour diffusants, because rapid cooling results in a greater profusion of fixed tie-molecules[6], which constrain long diffusants but have little effect on small ones. We report here results which support this model.

We measured D for two linear diffusants of the form $n-(CH_2)_NX$, X being a suitable label and $N \simeq 25$ or 45. The matrix was linear polyethylene cooled at two widely differing rates from the melt. The measuring technique was one we have recently developed, based on infrared microdensitometry[7], and involves setting up a known concentration distribution of diffusant within the host matrix, allowing it to broaden for a known time t at the required temperature, then measuring the resulting diffusion-broadened concentration profile. D can be evaluated knowing the shapes of the two profiles as well as t; Fig. 1 shows experimental profiles at the beginning and end of a typical diffusion run: the calculated curves (with the appropriate value of D) when superimposed on the experimental ones, show a good fit.

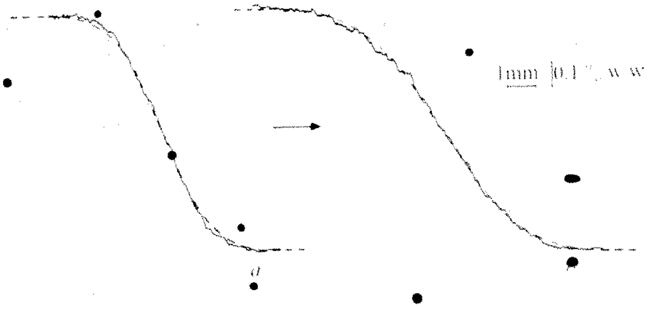

Fig. 1 Concentration–distance profiles as measured (—) at the beginning (*a*) and end (*b*) of a typical diffusion measurement. ----, calculated shapes as evaluated with the appropriate value of D. ($t = 1.29 \times 10^6$ s at 91 °C; the diffusant is a linear molecule of form $n-(CH_2)_{23}X$, X being an ester group; the matrix is linear polyethylene cooled rapidly from the melt, and the scanning frequency corresponds to the ester group absorption peak at 1720 cm^{-1}. D evaluated as 1.3×10^{-8} $cm^2 s^{-1}$.)

Table 1 summarises some of the results, giving D for the two diffusants in both rapidly and slowly cooled polyethylene matrix, and the corresponding values of the crystallinity x as derived from density measurements. In Table 2 we compare the prediction of the model (outlined above), which takes into account the topological constraints on the diffusants, with the experimental results (details of the calculations are given elsewhere[5]). The predictions of the simple two-phase relation, equation (1), are also shown.

Table 2 shows that agreement between observed and predicted ratios is fair to good. In particular, the model predicts correctly that D for the long diffusants used increases in the slowly cooled, more crystalline matrix—in contrast to equation (1).

The results provide support for the concept that long diffusants in semi-crystalline polymers may be constrained in a way which small diffusants are not; they suggest that studies involving such diffusants could yield information on the nature of the disordered phase in these polymers not otherwise available.

Table 1 D (in units of 10^{-9} $cm^2 s^{-1}$) for diffusants of form $n-(CH_2)_NX$* in linear polyethylene, at 84.8 °C

No. of backbone units N	Rapidly cooled matrix	Slowly cooled matrix
$N \simeq 45$	3.3 ± 0.4	6.5 ± 0.7
$N \simeq 25$	9 ± 1.8	11 ± 1.8
Crystalline volume fraction, x	0.751	0.855

*X is an ester group.

Table 2 Calculated and observed values of D for diffusants of form n-$(CH_2)_NX$ in linear polyethylene matrix at 84.8 °C

(a) Variation of D with cooling rate of matrix

No. of backbone units N:	D (slowly cooled matrix)/D (rapidly cooled matrix) Calculated	Observed	'2-phase', $D \propto (1-x)$
$N \simeq 25$	1.26	1.2 ± 0.3	0.58
$N \simeq 45$	1.52	2.0 ± 0.3	0.58

(b) Variation of D with diffusant length

	Rapidly cooled matrix Calculated	Rapidly cooled matrix Observed	Slowly cooled matrix Calculated	Slowly cooled matrix Observed
$\frac{D_{N\simeq 25}}{D_{N\simeq 45}}$:	2.18	2.7 ± 0.5	1.80	1.7 ± 0.3

Detailed accounts of both theory[1] and experiment[2] are in press.

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New transient effect observed in rock samples subjected to simulated upper crustal conditions

WHILE making laboratory measurements of the electrical conductivity (σ) of rock samples subjected to temperatures, confining pressures, and pore fluid pressures thought to be appropriate to depths up to 15 km within the Earth, we have observed a hitherto unreported transient behaviour which is only revealed by the simultaneous application of pressure and temperature, and leads to an irreversible decrease in σ with time which could be due to compaction of the rock samples. We suggest that only after such a change has occurred is the condition of rocks collected near the Earth's surface more representative of those at depth in the crust.

Most of the rock samples used in this study are high-grade metamorphics from the Lewisian of NW Scotland, which have a very low porosity (< 1%). These were cored, cut and ground into cylinders of diameter 24.5 mm and length 10–20 mm, and saturated with a 0.5 or 0.05 M sodium chloride solution. Two stainless steel electrodes allowed the a.c. conductance (normally at a frequency of 1.5 kHz) between the sample faces to be measured using a Wayne-Kerr B224 transformer bridge. Confining pressure acting on a rock within the Earth (due to the column of rocks above it) was simulated by hydrostatically pressurising the sample (up to 0.4 GPa (4 kbar)) in a pressure vessel. To prevent contamination from the oil pressure-medium the sample and electrodes were sheathed with a Teflon tube. It is believed that most rocks have sufficient strength to prevent their pores shutting completely under such pressure[1], and it is possible therefore for the pore fluid pressure to differ from the confining pressure. To allow for this, a hole passing through one electrode to the surface of the rock sample was connected to a pipe which passed out of the vessel, enabling the pore fluid pressure to be varied independently of the confining pressure. A furnace around the sample allowed temperatures up to 275 °C to be reached.

Because the bulk of the measured conductance in saturated rocks occurs through the solutions within the fine pore network, small changes in pore structure have a considerable effect on σ (refs 1, 2), and as σ can be measured accurately, it is probably the most sensitive property to use to investigate such small structural changes. Increasing the confining pressure closes up this pore structure, while increasing the pore fluid pressure has the opposite effect. It is reasonable therefore to assume a model in which the parameter that determines the overall pore structure, and therefore σ, is the pressure differential (known as the effective pressure) between confining and pore fluid pressure[3,4]. Most of our results at room temperature conformed to this model, with σ showing a steady decrease as the effective pressure was increased.

Figure 1 shows the typical behaviour observed when a sample at a fixed effective pressure was heated. From its room temperature value (a), σ increased along the path a–b as the temperature was increased. We found, however, that if the temperature was held steady at a high enough temperature (b), σ fell over a period of time to a lower value (c). This drop b–c did not occur suddenly at any specific temperature, and a slight drop has even been observed at temperatures as low as 130 °C, although at a very slow rate. Increasing the temperature has the effect of accelerating the rate, and samples were normally heated to around 200 °C or above, to enable us to observe a drop in a reasonable period of time. Figure 2a shows the time dependence

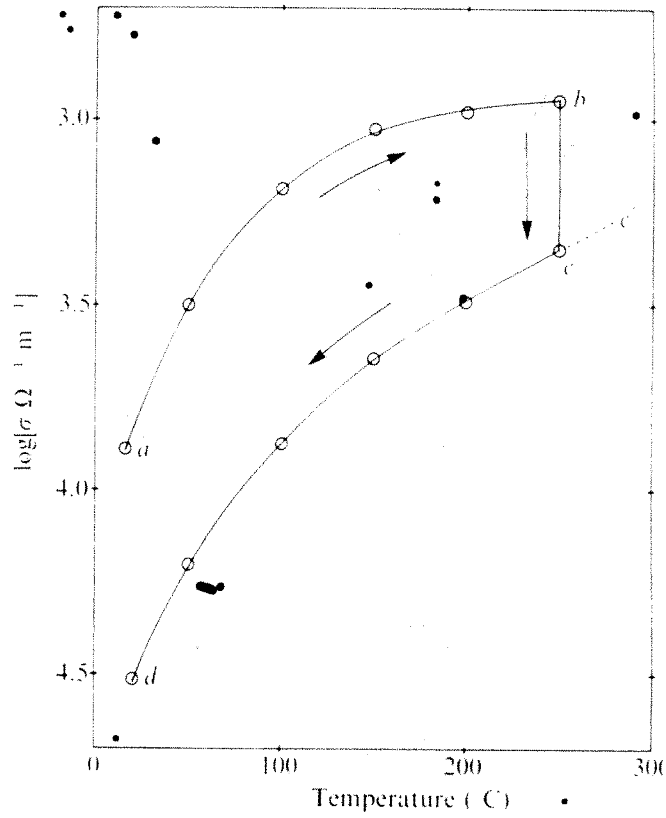

Fig. 1 Irreversible change in conductivity during the initial temperature cycle of a sample of gneiss saturated with 0.5 M NaCl solution and subjected to an effective pressure of 0.2 GPa (2 kbar).

of σ in one sample during the period of the drop. On cooling, the path c–d was followed. With any further increase or decrease in temperature, σ remained on the curve d–c, even when the temperature was increased beyond the point c to c', indicating that the rock had suffered some irreversible change. Although the rate of the drop varied from sample to sample, the general cycle *abcd* was observed in all saturated samples investigated. The feature does not seem to be confined to the very low porosity rock type used, since samples of basalt (2% porosity) and pyrophyllite (7% porosity) also showed a similar drop. Only in a sample pre-baked to remove any natural pore fluids was there no discernible drop in σ at high temperatures. Spurious effects, such as conduction paths between the rock and the Teflon sleeve, were discounted after making measurements with non-porous plugs (Teflon or sapphire) replacing the rock sample.

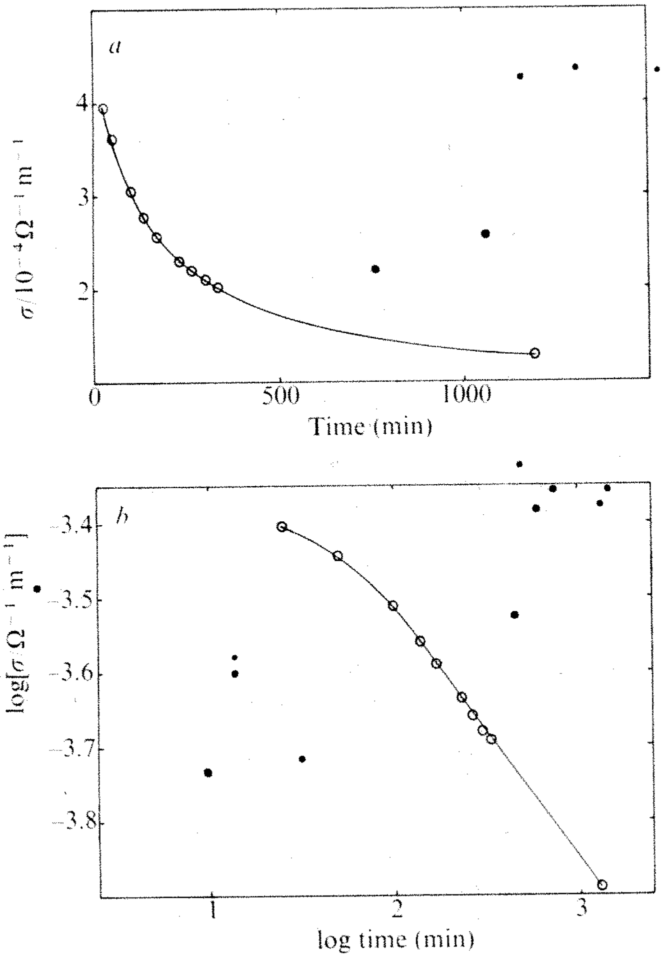

Fig. 2 Time dependence of the conductivity for a sample of granite gneiss (saturated with a 0.5 M NaCl solution) when subjected to an effective pressure of 0.35 GPa (3.5 kbar) at a temperature of 190 °C.

We believe a possible explanation for this behaviour is that in its initial state a, the sample contains many cracks and flaws associated with near-surface conditions such as weathering and unloading (and possibly with rock sampling and preparation)[5]. At room temperature, the strength of a rock is sufficient to prevent many of these cracks closing under pressure, but at elevated temperatures, the strength of a rock decreases, allowing more of the initial cracks to close and leaving the rock in a more compacted state. Figure 2b shows that apart from the initial period of the drop (where there is uncertainty in the precise onset), the variation of σ with time (t) may be expressed by the equation:

$$\log \sigma = A + B \log t,$$

where A and B are constants dependent on rock type, and the temperature and pressure conditions.

It has often been observed in transient creep measurements on rocks subjected to differential stress, that strain (ε) is also proportional to log t (refs 6, 7). If it could be shown, therefore, that a linear relationship exists between log σ and ε, it is possible that the drop we observe in log σ, when held at high pressure and temperature, may also be due to creep behaviour. Such a relationship might be argued as follows. In saturated rocks, it is found experimentally[1,2] that at moderate pressure (p),

$$\log \sigma \propto p.$$

Furthermore, from strain measurements made under such pressures[1],

$$\varepsilon \propto p.$$

Thus, it seems reasonable to assume that for any strain (whether elastic or inelastic)

$$\log \sigma \propto \varepsilon.$$

If this is the case (that is, that this transient behaviour is a sensitive indicator of a creep process), then presumably it is important to simulate this effect by thermal cycling of a rock sample under pressure before truly meaningful measurements of various physical properties of rocks can be made under laboratory conditions. To investigate this possibility, we are presently making simultaneous measurements of acoustic velocities and electrical conductivity in rocks, under the same conditions as described earlier, to examine whether the transient behaviour in σ reported here may also be observed in acoustic velocity measurements.

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Peridotite–CO_2–H_2O, and carbonatitic liquids in the upper asthenosphere

The recent discovery that CO_2 at upper mantle pressures has a dramatic effect on the melting temperature of peridotite (because the silicate assemblage becomes carbonated), and on the composition of near-solidus liquid, has petrological and geophysical implications that have been explored extensively. The influence of H_2O has received only passing reference, probably because it was tacitly assumed that this would modify the carbonate-dominated melting relationships significantly. I describe here construction of a schematic diagram for the system peridotite–CO_2–H_2O which shows that the carbonate can persist even in the

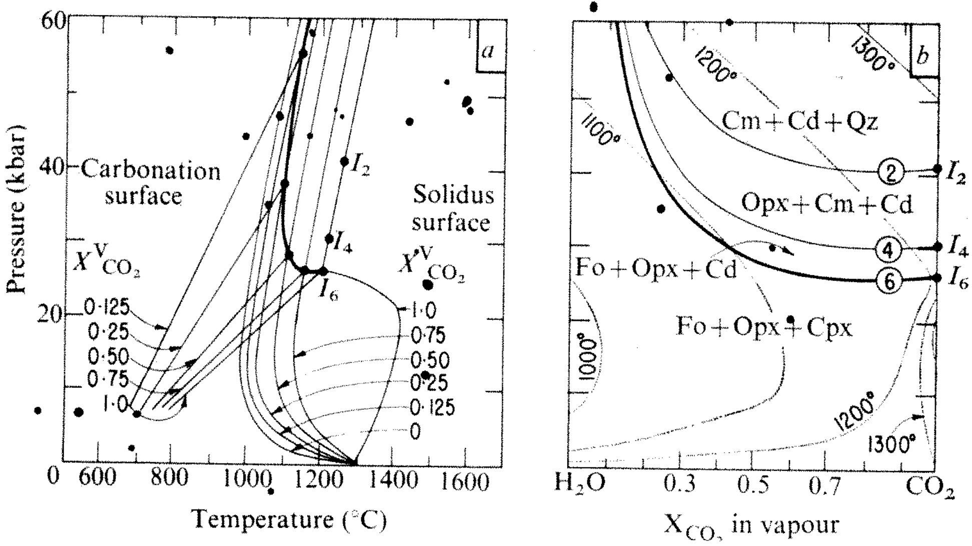

Fig. 1 Schematic phase diagram for the system peridotite–H_2O–CO_2, assuming a simple peridotite composition, Fo = 65%, Opx = 23%, Cpx = 12%. This illustrates the geometry for intersection of a divariant subsolidus carbonation reaction with the divariant solidus surface for melting in the presence of CO_2–H_2O vapours. It represents a compromise among several sets of experimental data, and precise pressures and temperatures need experimental determination (see ref. 21 for modifications of data from refs 5, 6, 10, 12, 13, 22). *a*, *P–T* projection. The univariant carbonation reaction extending to lower pressures and temperatures from I_6 is: (6) Cpx+Fo+CO_2 = Opx+Cd (calcite dolomite)[4–6]. In the presence of CO_2–H_2O vapours this reaction becomes a divariant surface extending to higher pressures and lower temperatures; it is mapped by contours for constant vapour-phase composition. Curves for the beginning of melting of peridotite in the presence of water[23] and CO_2[4,5,6] are connected by a curved surface, mapped in terms of contours for constant vapour-phase composition[10]. *b*, *P*–X projection (X is molar fraction). This shows the solidus surface, with estimated temperature contours. Vertical lines in this projection correspond to the contours in (*a*). The heavy line (6) corresponds to the intersection line of the two surfaces illustrated in (*a*). The lines (4) and (2) refer to other carbonation reactions[4,5] that are not encountered for reasonable mantle peridotite compositions, with low CO_2 contents. Mantle vapour-phase compositions are buffered to remain on the divariant carbonation surface of (*a*). Therefore at pressures (depths) greater than I_6, carbonate is stable in peridotite with total CO_2/H_2O ratios greater, at a given pressure, than the heavy line (6) in (*b*); similarly, vapour-phase compositions within the heavy line of (*b*) do not exist in the mantle at the pressures indicated. Abbreviations: Cm, magnesite solid solution; Qz, SiO_2 polymorphs.

presence of vapours with high H_2O/CO_2. Figure 1 shows that starting with peridotite–H_2O at high pressures, a small addition of CO_2 is sufficient for the generation of carbonate. This leads to the unexpected conclusion that many of the applications deduced for the melting of carbonated peridotite may remain valid with vapours enriched in H_2O/CO_2. In particular, the interstitial liquid in the upper part of the seismic low-velocity zone may be carbonatitic even with H_2O present as a component.

Until recently, it was believed that the solubility of CO_2 in mantle magmas remained low as it does in crustal magmas[1,2]. Experimental studies in silicate–CO_2 and silicate–carbonate systems have shown that peridotite becomes carbonated by the reaction Cpx+Fo+CO_2= Opx+Cd at high pressures[3–6] (Fo, forsterite; Cd, calcic dolomite; Opx, orthopyroxene; Cpx, clinopyroxene), and this causes a remarkable increase in the CO_2-solubility of near-solidus liquids[4,7,8]. The composition of the liquid produced by melting of the silicate–carbonate assemblage along the peridotite–CO_2 solidus rising from I_6 in Fig. 1*a* is 'carbonatitic' according to Wyllie and Huang[4,5,7], with CO_2 solubility approaching 40%, and 'kimberlitic' according to Eggler[6,8], with CO_2 solubility about 20%. Certainly, the CO_2 solubility is remarkably high, and the liquid composition is markedly different from that of normal basaltic magmas.

There is now convincing evidence that both H_2O and CO_2 do exist in the upper mantle[9–11] and it has been shown experimentally that the proportion of CO_2/H_2O influences the SiO_2 content of near-solidus liquids generated from mantle peridotite[10,12,13]. Figure 1 illustrates the effect of H_2O on the phase relationships for peridotite–CO_2; the geometrical relationships are correct although specific values for pressure and temperature need experimental determination.

It is assumed that the peridotite composition is not suitable for the stabilisation of amphibole and phlogopite, so that we can examine the influence of carbonate alone. Consider a partially carbonated peridotite composed of Fo+Opx+Cpx+Cd in the presence of a small amount of CO_2–H_2O vapour. This system must be situated on the divariant carbonation reaction surface and, at a given pressure and temperature, the vapour phase composition is given by the contour passing through the *P–T* point on the surface. With increase in temperature, decarbonation proceeds releasing CO_2 so that the CO_2/H_2O ratio in the vapour maintains the system on the surface. Similarly, carbonation occurs with increase in pressure, enriching the vapour in H_2O/CO_2. The principle is well established in metamorphic systems[14,15]; the vapour phase composition is buffered by the mineralogy.

The system can leave the surface for divariant reaction (6 in Fig. 1) only if carbonation or decarbonation are completed. The first alternative requires complete reaction of 12% clinopyroxene, which is most unlikely for any reasonable CO_2 content in mantle peridotite; therefore, the system cannot enter the high-pressure volume for complete carbonation in Fig. 1*a*, which meets the solidus surface in the area at pressures above the heavy line in Fig. 1*b*.

At a given pressure above I_6, the vapour phase in partially carbonated peridotite at its solidus temperature is constrained to lie on the solid line (6) in Fig. 1*b* (compare its position in Fig. 1*a*). For peridotite without crystalline carbonate, the vapour phase at the solidus temperature may have any composition at pressures below I_6, but it is limited to the higher H_2O/CO_2 ratios to the left of the heavy line (6) in Fig. 1*b*.

For the system peridotite–CO_2 at pressures above I_6, I am convinced that the near-solidus liquid is carbonatitic becoming kimberlitic with increasing temperature[4,5,7] (contrast Eggler). From the scanty data available in silicate–carbonate–CO_2–H_2O systems[16–18], I anticipate that the liquid composition at the solidus is dominated by the silicate–carbonate assemblage; any H_2O in the vapour dissolves in this liquid, with relatively little influence on the liquid composition. Accordingly, the liquid produced from the peridotite–carbonate assemblage could remain 'carbonatitic', even in the presence of H_2O/CO_2-enriched vapours. At pressures below I_6, near-solidus liquids become more siliceous with increasing H_2O/CO_2[10,13]. At pressures above

I_6, this is true also for the range of vapours coexisting with uncarbonated peridotite. Using the schematic phase diagram of Fig. 1 as a guide, we can reach the following conclusions.

For a partially carbonated mantle peridotite of fixed composition in thermal equilibrium, it can be determined from Fig. 1 that the amount of carbonate in the lithosphere decreases with depth, and the ratio of CO_2/H_2O increases in the vapour phase. If a portion of the mantle rises from the geotherm under adiabatic conditions, the amount of carbonate decreases, and the ratio of CO_2/H_2O in the vapour increases as the peridotite rises to shallower levels.

For a partially carbonated mantle peridotite, coexisting vapours have fairly high ratios of H_2O/CO_2 (Fig. 1*b*). Incipient melting begins with the generation of carbonatitic liquid. The liquid at the top of the seismic low-velocity zone may therefore be carbonatitic, with 10–15% dissolved silicates, Ca/Mg>1, and probably enriched also in alkalis and a host of trace elements. At greater depths, this grades into liquids with kimberlitic affinities. Carbonatitic liquids are very fluid, and they would probably migrate upwards. Local concentrations, and intrusions into the overlying peridotite, could produce a layer of lithosphere enriched in crystalline carbonates

This picture is consistent with a tentative proposal[19] that kimberlite magmas may be mixtures of silicate liquids from within the asthenosphere with carbonatitic liquids from near the top of the asthenosphere It is also consistent with the suggestion[20] that carbonatite magmas could be the fluids involved in the fluidisation emplacement of some kimberlites. For mantle peridotite with compositions appropriate for the formation of phlogophite, vapour phase compositions must be buffered by subsolidus reactions between phlogopite and carbonate in the presence of Fo+Opx+Cpx, which remain to be determined[21]. Alternatively, all CO_2 and H_2O may be stored in phlogopite and carbonate, with no free vapour phase.

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3-Gyr-old stromatolites from South Africa

WE report here a new record of early Precambrian stromatolites from the 3·0-Gyr Pongola Supergroup of South Africa (Fig 1), which is exposed in parts of the southeastern Transvaal, Swaziland, northern Natal and Zululand. These rocks occur in an almost complete section found in the Wit-Mfolozi River valley, about 200 km north of Durban. In that part of Natal the volcanic–sedimentary Pongola Supergroup attains a maximum thickness of about 2,500 m. It is subdivided into two distinct north-east-dipping stratigraphic units, the lower 1,800-m thick Insuzi Group and the upper 700-m Mozaan Group[1]. Bedding and primary sedimentary structures are ubiquitously well preserved Deformation is restricted to moderate cleaving of the argillaceous rocks. Evidence of regional metamorphism is restricted to incipient recrystallisation of the arenaceous and carbonate rocks; the lavas show evidence of deuteric alteration. Contact metamorphism is found where isolated diabase sills intrude the strata.

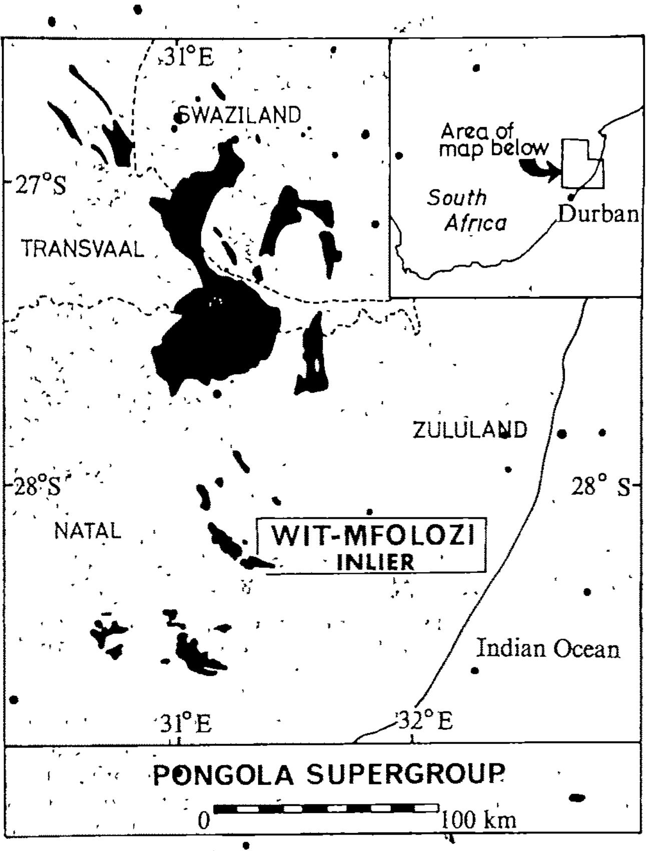


Fig. 1 The distribution of Pongola Supergroup exposures in the southeastern Transvaal, Swaziland, northern Natal and Zululand.

The Insuzi Group rests nonconformably[2] on crystalline basement rocks and comprises a repetitive sequence of quartz arenites and dolomites, with subordinate siltstone–mudstone units. The carbonates are of particular interest as no other recorded Insuzi Group exposures contain dolomites They vary in thickness (1–30 m), and are laterally impersistent, forming units in the succession which are only of local importance. Amygdaloidal lavas occur near the base and at the top of the Insuzi Group. The siliciclastic sediments provide evidence for deposition in a tide dominated environment, as they contain sedimentary structures including, lenticular, flaser and wavy bedding, mudclast conglomerates, herringbone cross lamination, and ripple marked bedding surfaces displaying desiccated mud drapes within their troughs Palaeocurrent data suggest that the coastline lay to the north. The unconformably overlying Mozaan Group is entirely composed of siliciclastic sediments of tidal origin and contains sedimentary structures similar to those noted in the Insuzi.

The stromatolites occur within Insuzi carbonates and resemble those recorded from the northern Slave Province (2·5 Gyr) of Canada[3] as they are generally of low relief, rarely rising more than a few cm above the contemporary substrate They form undulatory surfaces which are accentuated by the partial silicification of the host dolomite. Silicification was selective and not wholesale, so that instead of destroying the finer fabric of the rock this has been enhanced. The stromatolites commonly form low domes

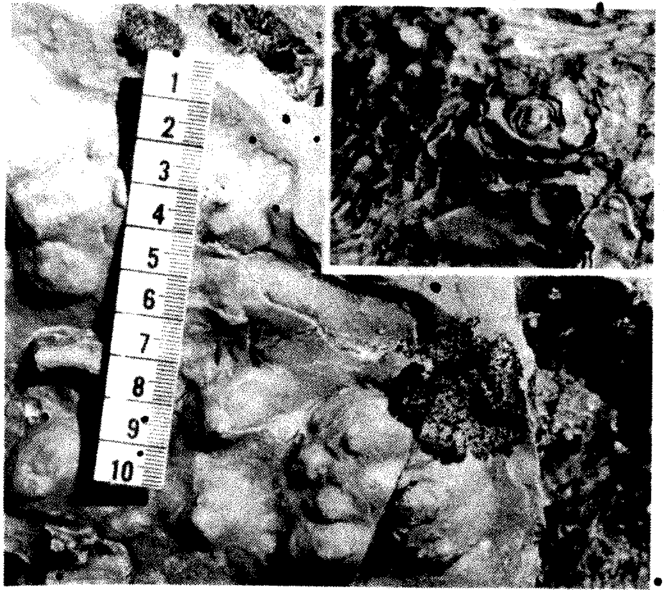
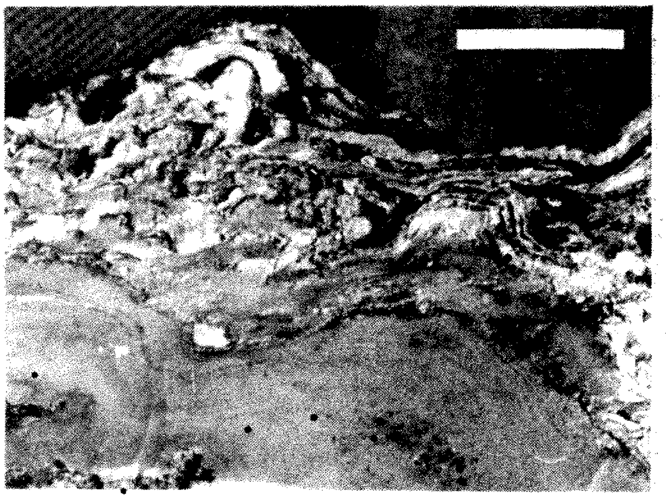


Fig. 2 Low relief domical stromatolites exposed on a bedding surface. Inset at same scale, exfoliated dome showing laminated structure.

(Figs 2, 3) and may be classified according to their geometry[4]. LLH-S, LLH-C and SH types have been recognised. The structures are thought to be of biogenic origin for a number of reasons. First, the preponderance of convex-upwards structures are strongly reminiscent of the growth habits of Recent algal communities. Second, the ubiquitous fine mm scale laminations in the rock are considered to be diagnostic of former algal growth[5], a conclusion reinforced by microscopic examination which reveals occasional fine sandy layers trapped between successive laminae (Fig. 4). A direct analogue of this feature may be found in the sediment trapping and binding habits of Recent blue-green algal mats[6]. Finally, some of the samples also display fenestral textures, with coarse sparry dolomite filling former voids in the rock. In Recent examples of algal sediments fenestrae are interpreted as structures formed by the interaction of algal mat and algal bound sediment. Desiccation, oxidation and lithification are also important factors leading to the formation of fenestral textures[7].

Characteristic sedimentary structures in the dolomites provide further evidence of shallow-water sedimentation. In the quartz arenites large dolomite clasts are found (about 30 cm) incorporated in the sandy sediment. They seem to have been reworked from adjacent carbonate mud flats by currents of considerable velocity. In addition, intraclastic dolomites suggest that there was significant penecontemporaneous reworking of the carbonates and, as these rocks contain herringbone cross lamination, tidal action was the most likely cause.

Fig. 3 Weathered surface showing a section through stromatolite structures. Note the differential erosion of the silica and carbonate layers, and the very fine lamination in the more massive silica at the bottom of the photograph. Scale bar 1 cm.



Insuzi Group stromatolites are of particular interest considering the scarcity of biogenic structures of this age in the geological record. Five comparable occurrences are documented, two in Canada[3,8] and three in Rhodesia[9,10]. The age of the Insuzi Group is not precisely defined. The overlying Mozaan Group rests with a sedimentary contact on the 3.06-Gyr (Rb/Sr) Lochiel granite of the southeastern Transvaal[11], and is intruded by the Usushwana Complex dated at 2.87 Gyr (ref. 12). Zircons from the Insuzi lavas at Nkandhla gave an age of 3.09±0.09 Gyr (ref. 13) and a whole rock Rb/Sr age of 3.15±0.15 Gyr is reported from Insuzi felsites[13] in the Mozaan valley of Swaziland. From the foregoing evidence there seems little doubt that the Insuzi Group is approximately 3.0-Gyr-old. Comparing this date with the ages generally quoted for the other five localities we find that the age of the stromatolites at Steeprock, Ontario, is in dispute, while those of the northern Slave Province[3] (2.5 Gyr) post-date the Pongola. Work on the Rhodesian greenstone belt[10,14,15] suggests that the Bulawayan and Belingwe stromatolites are about 2.6–2.7-Gyr-old. Assuming that this age is correct, and that it does not represent the resetting of the radiometric clocks by the most recent metamorphic event, the Insuzi stromatolites are the oldest so far documented.

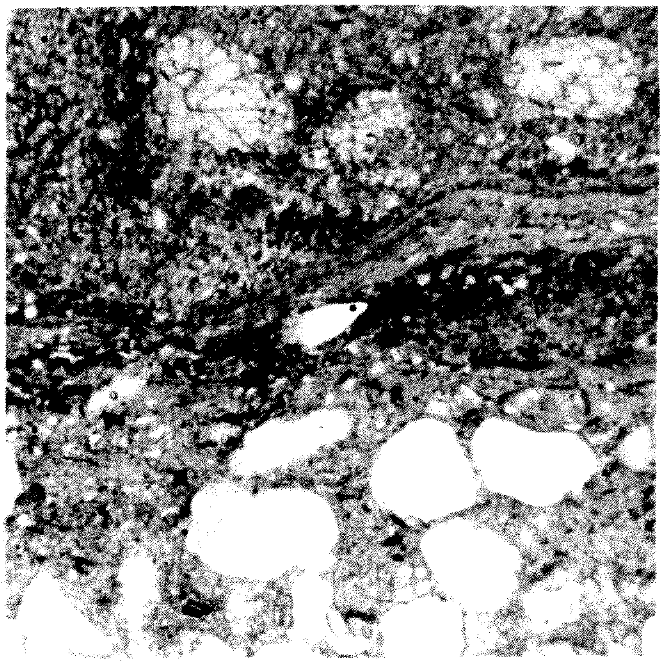


Fig. 4 Fine sandy layer trapped within laminated stromatolite. Note sparry dolomite filling former voids in the rock. Magnification ×70.

Considering the criteria of emergence and shallow water sedimentation found in the quartz arenites in the Wit-Mfolozi section and the evidence for marine deposition provided by the stromatolites, there is little doubt that the Insuzi Group was deposited in a tidally influenced environment. Von Brunn and Hobday[16], applying Klein's[17] model to other Pongola exposures in this region, concluded that the palaeotidal range lies between 12 and 25 m. In the area under discussion comparable palaeotidal amplitudes are suggested by similar sedimentary sequences. Such large amplitudes may have relevance to hypotheses concerning the capture of the Moon by the Earth. The relationship between the stromatolite-bearing carbonates and evidence of extensive volcanicity is also of note. In all the other early Precambrian occurrences, stromatolites are associated with lavas, and we speculate that the exhalative activities of lava extrusions provided optimum conditions for stromatolite growth. The Earth's atmosphere at the time was probably anaerobic[18], and some Recent algal species can thrive in such an environment. If ancient stromatolites produced oxygen by photosynthesis, this could have been immediately removed from the environment as free oxygen, and transported to one of the oxygen sinks then operative. The thin banded ironstones in the overlying Mozaan might owe their presence to the local production of biogenically derived oxygen during those times.

Cloud[19] adapted many of his former ideas concerning the major features of crustal evolution to account for the anomalous presence of banded ironstones and stromatolites in the Pongola. He suggested that the events taking place in southern Africa in the early Precambrian were out of phase with those of the rest of the planet in that they anticipated many later developments in other areas.

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Pigmentation of the ladybird beetle *Coccinella septempunctata* by carotenoids not of plant origin

MANY insects contain carotenoid pigments[1], and some notable examples are found in the Coleoptera, where carotenoids are responsible for the yellow colour of the yellow and black striped wing cases of the Colorado beetle, *Leptinotarsa decemlineata*, and the orange-red colour of the ladybird beetles, *Coccinella* spp. There has, however, been no report of any insect species being able to synthesise carotenoids *de novo*, and it is generally accepted that in insects, as in other animals, any carotenoids present are of dietary origin[2]. In July 1976 the Liverpool area, like many regions of the UK, experienced an invasion by enormous numbers of ladybirds, almost entirely of the seven-spot variety, *Coccinella septempunctata*. With such large numbers of insects available we were able to use modern analytical techniques to identify the carotenoids of these beetles. We report here that the carotenoids identified were mostly hydrocarbons, and the carotenoid pattern indicated that these pigments in the ladybird are likely to be of microbial rather than plant origin, thus suggesting the involvement of symbiotic microorganisms.

Preliminary thin-layer chromatographic analysis showed that the carotenoid composition of isolated red wing cases (elytra) was the same as that of the entire insect, so whole insects were used in the large-scale analyses. Carotenoid hydrocarbons formed the bulk of the pigment, but there were also small amounts of many xanthophylls, of which only lutein (β, ε-carotene-3,3′-diol) was identified with certainty. The carotenes were characterised by chromatographic properties, light absorption and mass spectra, and whenever possible by comparison with authentic samples. Eighteen compounds were identified, including β-carotene (β,β-carotene), previously reported by Lederer[3] and by Valadon and Mummery[4]. One of the main carotenes present was torulene (3′,4′-didehydro β,ψ-carotene), and smaller amounts of γ-carotene (β,ψ-carotene), β-zeacarotene (7′8′-dihydro-β,ψ-carotene), 'cyclic ζ-carotene' (7′,8′,11′,12′-tetrahydro-β,ψ-carotene), lycopene (ψ,ψ-carotene) and 3,4-didehydrolycopene (3,4-didehydro-ψ,ψ-carotene) were present. A second series of compounds present possessed a methylenecyclohexane ring system (γ-ring) (ref. 5), a very unusual structural feature. β,γ-Carotene, γ,γ-carotene, γ,ψ-carotene, 3′,4′-didehydro-γ,ψ-carotene, 7′,8′-dihydro-γ,ψ-carotene and 7′,8′,11′,12′-tetrahydro-γ,ψ-carotene were all identified. The natural occurrence of the last three of these carotenoids has not been reported previously. A series of more saturated acyclic compounds that are intermediates in carotenoid biosynthesis[6] was identified—phytoene (7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene), phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene), neurosporene (7,8-dihydro-ψ,ψ-carotene) and 'ζ-carotene' (a mixture of symmetrical 7,8,7′,8′,-tetrahydro-ψ,ψ-carotene and unsymmetrical 7,8,11,12-tetrahydro-ψ,ψ-carotene). The carotenoids present in the largest amounts were torulene and β,β-, β,γ-, β,ψ- and γ,ψ-carotenes.

Phytoene, phytofluene, 'ζ-carotene', neurosporene, 'cyclic ζ-carotene' and β-zeacarotene are possible intermediates (as are lycopene and γ-carotene) in the biosynthesis of β-carotene, and have not been detected before in any insect or other animal. The presence of lycopene in the ladybird has finally been proved, long after evidence for its occurrence was first reported[3]. The γ-ring carotenoids and the carotenes of the torulene series are also unusual and their presence in an animal is surprising.

The question of the origin of these pigments cannot be answered in terms of the accepted view that insect carotenoids are of dietary origin[2]. The food chain involving the ladybird is short and simple. The beetles feed on aphids which in turn feed on the juices of green plant tissues. Within this food chain only the green plants are known to be capable of synthesising carotenoids, and there is no known metabolic process whereby the bicyclic compounds with β- and ε-rings, produced by the plants, could be transformed into acyclic, monocyclic or γ-ring carotenes. The ladybird carotenes must therefore originate elsewhere.

The γ-ring carotenes, β,γ-carotene and γ,γ-carotenes, together with β-carotene have, however, been isolated from a green variant of the aphid, *Macrosiphum liriodendri*, whereas pink variants of the same species contained β-carotene, γ-carotene, torulene, lycopene and 3,4-didehydrolycopene[7]. We stress that the ladybirds used in our investigation were members of a huge, migratory, starving population, and nothing was known of their previous food intake. Although it is unlikely that the ladybirds or their larvae would have had access to the aphid species *M. liriodendri*, which seems to be a specific parasite of the tulip tree (*Liriodendron tulipifera*), it is not unreasonable to expect that the γ-ring and torulene series carotenoids may be present in other species of aphid which may have formed part of their diet. Even so, the aphids themselves could not obtain these carotenes from the green plants on which they feed.

We therefore conclude that these carotenes are synthesised within the aphid and/or the ladybird. The possibility of synthesis *de novo* by the insect cannot be ruled out, although this would be the first instance to be discovered of carotenoid synthesis by any animal. The involvement of symbiotic microorganisms seems more probable, and the nature of the carotenoids present gives indirect support for this theory. Apart from these reports of their occurrence in the insect species, γ-ring carotenoids have been found only in a discomycete fungus *Caloscypha fulgens*[8]; torulene and related compounds are the characteristic pigments of red yeasts (*Rhodotorula* spp.)[9]. Further, the 'ζ-carotene' isolated from ladybirds was a mixture of the symmetrical and unsymmetrical isomers, a situation encountered commonly in bacteria and fungi but not normally found in higher plants[10]. Weisgraber *et al.*[11] have summarised evidence which they believe suggests strongly that the γ-ring and torulene series carotenes present in the aphid *M. liriodendri* are of microbial origin, and aphids in general are rich in symbionts (yeasts, bacteria, etc.) some of which are red[12].

For the ladybird, many of the carotenes present could presumably have been derived from aphids (or their symbiotic microbes) devoured by the beetles, but it seems unlikely that phytoene and the other desaturation and cyclisation intermediates would be absorbed and accumulated by the ladybird if they were obtained only in minute amounts in its food Their presence suggests a site of synthesis within the ladybird itself. Although no information is available about the microflora of the ladybird, it is reasonable to believe that carotenogenic microorganisms present in its aphid prey could be acquired and cultivated by the beetle. Thus it is likely that the carotenoids responsible for the characteristic red colour of *C. septempunctata* are synthesised within the insect, probably through an association with symbiotic microorganisms. Preliminary analysis indicates that the situation may be similar in other species of ladybird.

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Naturally occurring viral R plasmid with a circular supercoiled genome in the extracellular state

PHOTOSYNTHETIC bacteria have been used extensively in studies on photosynthesis, photochemical nitrogen fixation and photoevolution of hydrogen A major obstacle to combined biochemical and genetic studies of these processes is a lack of knowledge of gene transfer in such bacteria. The only transfer mechanism known is that reported by Marrs for the facultative phototroph *Rhodopseudomonas capsulata*[1]. In this bacterium gene transfer is mediated by a filterable agent of small size (Gene-Transfer-Agent) estimated to carry between 10^5 and 10^6 daltons of DNA; the nature of these GTAs is unknown Although GTAs have been used to construct a preliminary map of the bacteriochlorophyll and carotenoid biosynthetic genes in *R. capsulata* (ref 2), the small amount of DNA transferred limits their usefulness in genetic analysis To obtain alternative means of gene transfer we examined isolates of the closely related species *R. sphaeroides* for temperate transducing bacteriophage and conjugative R plasmids.

Several temperate, but evidently non-transducing bacteriophage have been isolated from *R. sphaeroides* (ref. 3) and *R capsulata* (ref. 4) Many of our wild type isolates carry between one and three prophages and more than 90% are resistant to penicillin because of a diffusible penicillinase (unpublished data) Since the production of diffusible penicillinases by *Staphylococcus aureus*[5], *Pseudomonas aeruginosa*[6] and various enteric bacteria[7] is encoded by plasmid genes, extrachromosomal DNA isolated from *R. sphaeroides* (refs 8, 9) could constitute extrachromosomal prophages and/or penicillinase plasmids.

Initial experiments with a penicillinase-producing strain of *R sphaeroides* RS601 (prototrophic, penicillin resistant) showed that a sensitive mutant RS602 (prototrophic, penicillin sensitive), isolated after treatment with the mutagen nitrosoguanidine, was 'cured' of a prophage. Bacteriophage released spontaneously by the wild type RS601 (10^5–10^6 plaque-forming units per ml of supernatant) produced small (1 mm diameter) turbid plaques on the 'cured' derivative RS602.

Although attempts to perform generalised transduction with this bacteriophage (designated Rφ6P) were unsuccessful, when recipient cells (RS602) were grown in aerobic conditions at 32 °C and at a multiplicity of infection of 1.0, 50% of 'survivors' of bacteriophage infection were penicillin resistant. An analysis of 100 penicillin resistant transductants derived from this experiment showed that each produced a diffusible penicillinase

The question now arises as to the exact nature of this high frequency transduction; at least two explanations seem possible Either the transduction is mediated by a defective specialised transducing bacteriophage analogous to PIIde in *Staphylococcus aureus*[10], or the wild type bacteriophage

Table 1 The relationship between penicillin resistance and lysogenisation by Rφ6P in *R sphaeroides*

Possible phenotypes	Number of colonies
$\mathrm{Pen^s}\,\varphi^-$	1127
$\mathrm{Pen^s}\,\varphi^{+}$	0
$\mathrm{Pen^r}\,\varphi^-$	0
$\mathrm{Pen^r}\,\varphi^+$	123

An exponential culture (about 5 × 10^8 colony forming units per ml) of RS602 (penicillin-sensitive, Rφ6P sensitive) grown with aeration at 32 °C, was infected with Rφ6P at a multiplicity of 0.1 The bacteriophage were allowed to adsorb for 30 min at 32 °C without aeration and then excess free bacteriophage were removed with anti-bacteriophage antiserum. The bacteriophage infected cells were diluted and plated on PYEA (0.3% peptone, 0 3% yeast extract, 1.5% agar) to give approximately 50 colonies per plate. After 2 d of incubation at 32 °C the colonies were patched on to PYEA plates and later replicated onto PYEA + 1 μg ml^{-1} penicillin to determine their resistance/sensitivity phenotype. Lysogeny, as determined by release of free bacteriophage, was assayed by overlaying the patch plates with soft PYEA (0.8% agar) seeded with RS602 carries a gene for penicillinase production. In experimental conditions which limit infection to a single bacteriophage per cell, there was a 100% correlation between the presence of wild type bacteriophage and production of penicillinase (Table 1). Hence the penicillinase gene is probably carried as part of a wild type bacteriophage genome.

In view of the importance of plasmid coded penicillinase activity we explored the possibility that Rφ6P exists as a plasmid in the lysogenic cell. A simple test used to differentiate plasmid and chromosal genes is the Arber experiment[11]. Low level ultraviolet irradiation of transducing bacteriophage lysates stimulates transduction of chromosomal genes and depresses transduction of plasmid genes. Figure 1 shows a depressed frequency of transduction following irradiation, indicative of an extrachromosomal location for this gene in the transduced (lysogenic) cell. Confirmation on these data is being sought by the isolation and characterisation of plasmid DNAs from lysogenic (Rφ6P+) and non-lysogenic (Rφ6P−) derivatives of *R. sphaeroides*.

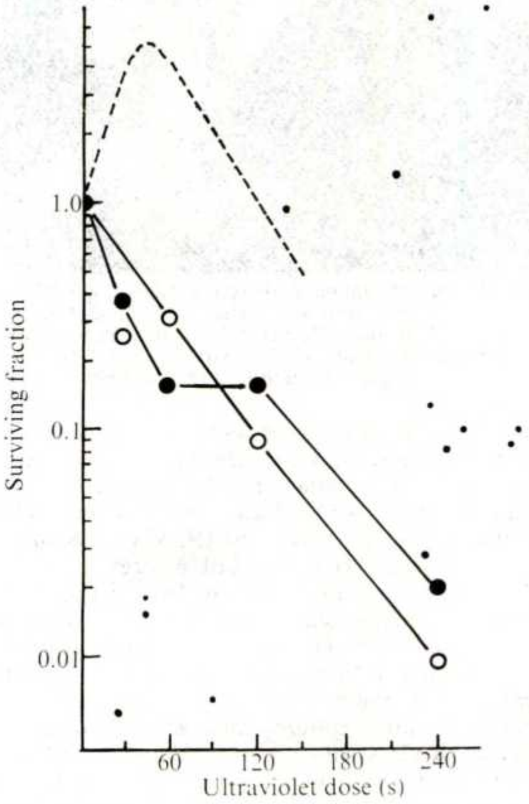

Fig. 1 The effect of ultraviolet-irradiation on the transduction of the penicillinase gene by Rφ6P. Bacteriophage Rφ6P was diluted in bacteriophage buffer (ref. 3 to give 10^9 plaque-forming units per ml and was ultraviolet irradiated for various lengths of time. Plaque forming titre (○) and transducing titre for the penicillinase gene (●) were determined for each dose of ultraviolet irradiation. The broken curve illustrates the effect of ultraviolet radiation on transduction of chromosomal markers in *Escherichia coli* (ref. 11).

It is unlikely that transforming DNA or gene-transfer-agents are involved in this process because transfer is insensitive to DNAase; inactivated by anti-Rφ6P antisera; and the vector has a molecular weight consistent with the purified bacteriophage particle. An electronmicrograph of the Rφ6P particle (Fig. 2) shows an isometric head 65 nm in diameter with a long flexuous non-contractile tail 12 nm × 250 nm, 2–4 tail fibres and no prominent tail plate. DNA molecules obtained from the bacteriophage by phenol extraction[12] and prepared for electron microscopy by the Kleinschmidt technique[13,14], show a majority of supercoiled molecules suggesting the encapsidated viral genome is circular. Using a variety of means such as DNase, freezing and thawing, alkali and heat treatment, the supercoils could be 'relaxed' to give open circles having a molecular length of $16.5\pm1.0\ \mu m$ which corresponds to a molecular mass of $33\pm2\times10^6$ (ref. 15). The only other report of a bacteriophage which possesses a closed circular, supercoiled, extracellular genome is PM2, a bacteriophage whose natural host is a marine pseudomonad[16].

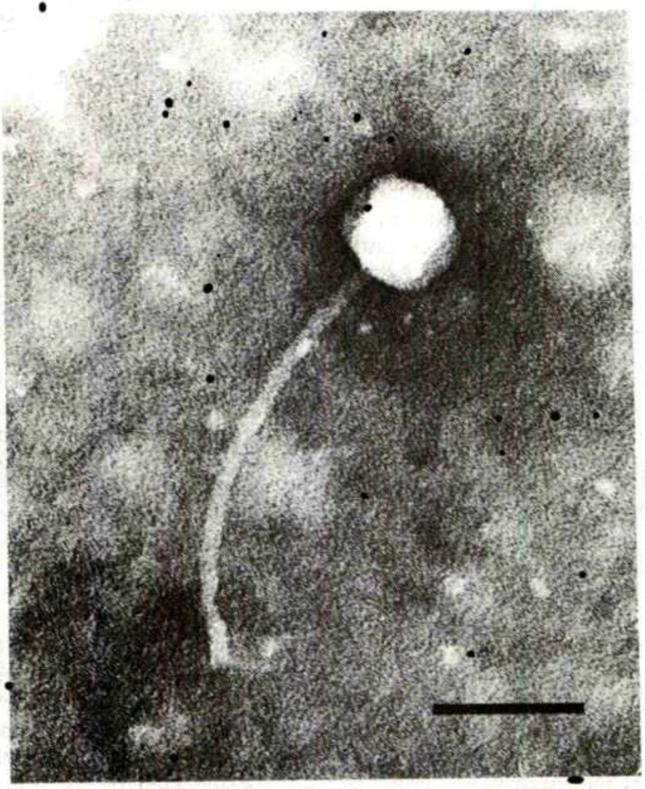

Fig. 2 Bacteriophage Rφ6P negatively contrasted with ammonium molybdate 1.0% (*p*H 6.0) using the procedure of Miller, Pemberton and Richards[17]. The bar represents 100 nm.

Richmond[7] states that "purely on statistical grounds bacteriophage conversion and restricted transduction are more likely to play some part in the spread of antibiotic resistance markers than is generalised transduction". This paper records the first instance of naturally occurring phage conversion involving an antibiotic resistance gene. If such virus-R plasmid combinations are widespread in nature then antibiotic therapy directed against drug resistant bacteria may be ineffective in removing the extracellular form of the plasmid from the environment. Finally, R-plasmids possess genes whose functions are still unknown; is it possible that some of these genes encode viral functions?

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Early presence of ribonucleoprotein antigen on surface of influenza virus-infected cells

HOST defence against influenza A virus in experimental animals seems to be mediated mainly by antibody[1,2]. The mechanism by which antibody protects the host, however, is unknown. Antibody may either neutralise viral particles or recognise viral antigens at the surface of infected cells and then act at an early stage of infection, before viral replication is completed. The latter mechanism is theoretically more efficient, but might cause immunopathological destruction of the infected cells with harmful consequences. Viral envelope antigens (haemagglutinin and neuraminidase) have been shown to be present at the surface of influenza virus-infected cells, by haemadsorption[3], electron microscopy[4] and immunofluorescence[5]. Two main type-specific internal antigens have been described in influenza A virus—the matrix protein (MP) antigen[6] and the ribonucleoprotein (NP) antigen[7], a non-glycosylated polypeptide with a molecular weight of 58,000 (ref. 8). Immunofluorescence studies on fixed cells have shown that NP antigen is first demonstrable in the cell nucleus and then appears as granular masses within the cytoplasm, which become prominent at the cell borders later in infection, suggesting a close association with the cell membrane[9]. We report here that NP antigen can be detected by immunofluorescence as early as 2 h after the infection on the surface of unfixed mouse cells infected with influenza A virus.

Two methods were used to obtain anti-NP antibodies. The first is described in Table 1. A pool of sera was collected from CBA mice infected intranasally 3 weeks previously with a sublethal dose of an egg-grown A/PR/8 virus. After two cycles of absorption with the homologous concentrated A/PR/8 virus, no detectable antibodies to external components of the virus could be found in this serum, as shown by its negativity in single-radial-diffusion test (SRDT) immunoplates containing intact A/PR/8 virus particles as antigen. The zone area obtained in a SRDT immunoplate containing a disrupted influenza A avian virus (external antigens unrelated to those of A/PR/8 virus), however, was only slightly decreased. Thus, antibodies to internal components of all influenza A viruses were detectable in the serum before and after absorption. At low concentrations of disrupted avian influenza A antigens, the SRDT method reveals exclusively anti-MP antibodies when an anti-human influenza A serum is tested[10]. Comparison of results in SRDT with high and low concentrations of avian influenza A antigens indicated that anti-NP, but not anti-MP antibodies, were detectable in this serum. The second method to obtain anti-NP antiserum from rabbits immunised with NP antigen isolated after electrophoresis of influenza A/Hong-Kong/68 (H3N2) on cellulose acetate paper strips has been described before[11].

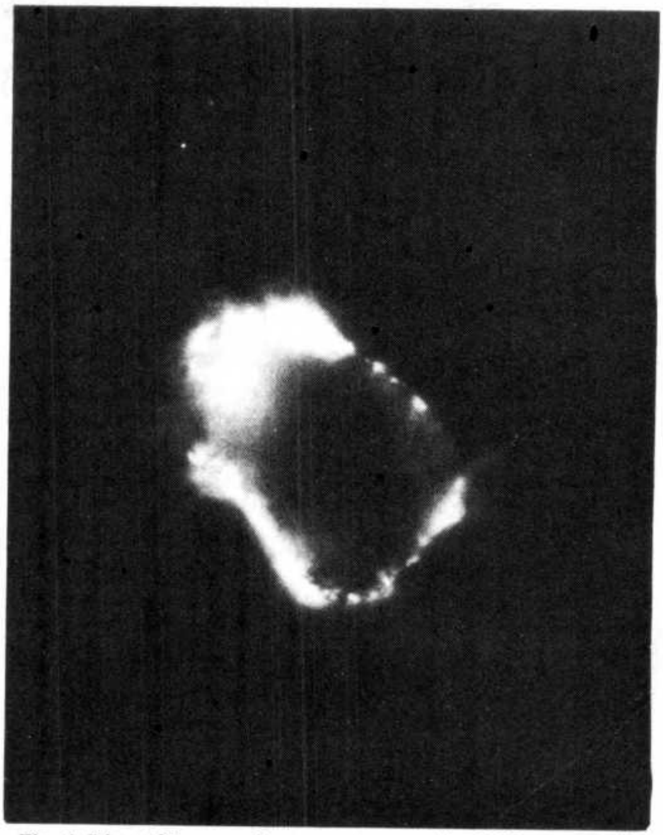

Fig. 1 Ring of immunofluorescence around a D55 mouse cell infected 3 h previously with influenza A/turkey/England/63 and treated with an anti-influenza A NP antigen (mouse anti-A/PR/8 serum absorbed with A/PR/8 particles) and FITC conjugated goat anti-mouse immunoglobulins.

For indirect surface immunofluorescence, unfixed, infected D55 mouse cells adherent on glass coverslips were treated as described in Table 2. Uninfected cells or infected cells incubated with fluorescein isothiocyanate (FITC) conjugated anti-immunoglobulins sera alone were negative. Similarly, control cultures infected with B/Lee virus (an influenza B virus with no cross reactivity with external or internal components of influenza A viruses) were negative.

Table 1 Specificity of anti-influenza antibodies in a mouse convalescent anti-A/PR/8 serum after absorption with A/PR/8 virus

Antigen in SRDT immunoplate*	Specificity of antibodies detectable with immunoplate	Zone area before absorption (mm²)	Zone area after absorption with A/PR/8 virus† (mm²)
Purified-concentrated A/PR/8 virus (15 mg ml⁻¹ in agar)	Anti-external antigens (haemagglutinin + neuraminidase)	[illegible].0	0
Purified-concentrated and SSS detergent disrupted avian N virus (1.5 mg ml⁻¹ in agar)	Anti-internal antigens (NP + MP)	10.7	8.8
Purified-concentrated and SSS detergent disrupted avian N virus (0.15 mg ml⁻¹ in agar)	Anti-MP	0	0

*A/PR/8 antigens were intact whole particles. The A/chicken/N/Germany/49 (Hav 2 Neq 1) was disrupted by mixing with equal volume of 1% sodium-sarkosyl sulphate (SSS) for 5 min before being mixed with 3 ml of agar.

†Equal volumes of anti-PR8 serum and of a purified-concentrated suspension of A/PR/8 virus (10 mg viral proteins per ml) were incubated for 1 h at 4 °C and centrifugated at 100,000g for 1 h. This procedure was repeated twice. The final supernatant was absorbed twice with packed chicken erythrocytes and heated at 56 °C for 1 h to remove all remaining viral particles. At the end of the process, an haemagglutination test was negative ($<\frac{1}{2}$).

Table 2 Surface immunofluorescence detected by anti-NP antibodies in mouse cells infected with influenza virus

Infecting virus	Mouse anti-A/PR/8 serum absorbed by A/PR/8 virus			Rabbit anti-NP serum			Haemadsorption			
	2 h	3 h	4 h	2 h	3 h	4 h	2 h	3 h	4 h	12 h
B/Lee	0	0	0	0	0	0	0	0	*	***
A/PR/8	0	0	*	0	0	*	0	0	0	*
A/Turkey/Eng.	*	***	**	*	***	**	0	0	*	***

Non-confluent monolayers of cells from the D55 mouse permanent cell line adherent on glass coverslips were infected with influenza B/Lee, A/PR/8/34 (HONI) or A/Turkey/England/63 (Hav 1 Nav 2) at about 100×ID50 per cell. At various intervals after infection the coverslips were washed thoroughly in cold phosphate-buffered saline (PBS). The cells were not fixed. After 30 min incubation at 4 °C with either inactivated mouse anti-A/PR/8 serum absorbed with A/PR/8 virus (see Table 1) or a rabbit serum anti-purified NP from A/Hong-Kong/68, the cultures were incubated for a further 30 min with (respectively) FITC-conjugated goat anti-mouse immunoglobulins serum or swine serum anti-rabbit immunoglobulins. After 1 h washing in cold PBS, they were mounted on glass slides with 50% glycerol phosphate. Surface immunofluorescence was recorded as absent (0), slight (*) intense (**) or very intense (***). Haemadsorption was performed by adding a suspension of 1% chicken erythrocytes for 30 min at 4 °C followed by washings in PBS.

This was not due to lack of production of influenza B antigens since B/Lee infected cells were able to haemadsorb chicken erythrocytes as early as 4 h after infection, and gave an intense haemadsorption 12 h after infection. A bright, 'patchy' immunofluorescence was found all over the surface of cells infected with either A/PR/8 or A/turkey virus (Fig. 1). This immunofluorescence was detected as early as 2 h after infection in A/turkey infected cells, increased rapidly and was maximal 3 h after infection, when the pattern was a complete ring of fluorescence around the cells. There was no evidence of capping. The fluorescence began to fade 4 h after infection, when the first haemagglutinins were appearing on the surface as shown by the slight positivity of the haemadsorption test at that time. The fluorescence was weaker and appeared later in A/PR/8 than in A/turkey infected cells. The negativity of haemadsorption at 4 h, and the modest positivity of haemadsorption found at 12 h suggest that A/PR/8 virus was poorly infective in D55 cells; this could explain the more discrete immunofluorescence found in these cells. Surface immunofluorescence was also detected with a rabbit anti-NP antiserum in primary kidney cells, Vero cells and MDBK cells infected with influenza A/FPV, A/NWS and A/HK/1/68 virus.

Our results strongly suggest that NP antigen is present on the surface of cells infected *in vitro* with influenza A virus. Thus two anti-NP sera gave a fluorescence on unfixed cells infected with an avian virus with external antigens unrelated to those of the viruses used to raise the antisera. But, two more internal structural polypeptides have been described in influenza A virus, P1 and P2 (ref. 8). The possibility that P1 and P2 may be present as minor contaminants of the NP antigen as separated on cellulose acetate strips cannot be excluded. At present, there is no way to detect selectively anti-P1 or P2 antibodies. The presence of such antibodies in our convalescent mouse anti-influenza serum, however, seems unlikely. Finally, non-structural antigens induced by influenza A virus have been detected by immunofluorescence in nucleoli of infected cells[12], and two non-structural viral polypeptides have been described[13]. If we fixed our cells with acetone, however, NP antigen was detected in the nuclear and paranuclear area of the cells, but there was no immunofluorescence in the nucleoli. The presence of antigens related to the virus core on the cell surface is not confined to influenza virus, since analogous observations have been made with oncornaviruses[14]. Presence of these antigens early in infection may facilitate assembly with the envelope antigens which enter in the membrane later. Pulse labelling studies with ^{35}S-methionine have demonstrated the early synthesis of the NP polypeptide in chicken embryo fibroblast cells infected with fowl plague virus and the association of this protein with membrane fractions of the infected cell[15]. The relevance to host defence mechanisms of our finding that NP antigen is present at an early stage on the surface of influenza virus-infected cells is not known. In theory, this would allow antibody or lymphocytes with anti-NP specificity to destroy infected cells at a stage when the virus has not yet replicated. Since all influenza A viruses share the same NP antigens, such a protective mechanism might cross the subtype barrier. Indeed, our unpublished results, and other reports[16,17] suggest that cross protection can be found between influenza A viruses which do not cross react at the level of their external antigens. The presence of type-specific internal antigens on the surface of infected cells might explain these observations. However, we have so far been unable to protect mice or ferrets by immunisation with purified MP or NP antigens or by passive immunisation with anti-NP or anti-MP antibodies[11,18]. This failure may mean either that the presence of NP-antigen on the surface of infected cells is not relevant to host defence, or that our immunisation technique was not sufficient to elicit an efficient immune response. Indeed a failure to induce protection by passive transfer of anti-NP antibody cannot exclude the possibility that, under physiological conditions, lymphocytes, but not antibody, can recognise NP antigen on the cell surface and protect the host either by direct cytotoxicity or by lymphokine secretion. Further work is needed to determine whether internal viral antigens on the surface of infected cells can be responsible for the paradoxical heterotypic protection observed with influenza A virus.

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Infectious DNA from a whitefly-transmitted virus of *Phaseolus vulgaris*

GOLDEN yellow mosaic (bean golden mosaic) is a widespread disease of *Phaseolus vulgaris* in tropical and subtropical America[1]. The disease causes serious yield losses in several countries where beans are an important dietary staple[2,3]. The causal agent, long presumed to be a virus, also infects *P. lunatus* and *Macroptilium lathyroides* in the American tropics[4,5]. The pathogen is transmitted in nature and in the laboratory by the whitefly *Bemisia tabaci* Genn., and is also mechanically transmissible from the sap of infected plants[3,5]. An unusually small (18-nm diameter) nucleoprotein particle has been purified from diseased plants[5,6], and shown conclusively to be the whitefly-transmitted virus causing the disease[5]. Electron microscopy of purified virus revealed predominately geminate aggregates of the 18-nm particles[5,6]. The virus must be aldehyde-fixed before negative staining because it is disruped by the heavy metal salts (ammonium molybdate, sodium phosphotungstate, or uranyl acetate) used as negative stains. Thus, it is not known whether the geminate aggregate is the infectious entity or an artefact of fixation[5]. Nonetheless, its unusually small size sets this virus apart from most other plant viruses. I describe here the extraction of infectious DNA from purified golden yellow mosaic virus (GYMV), a result that further separates this virus from other previously described plant viruses.

Highest yields of purified virus were obtained from the leaves of virus-infected plants (*P. vulgaris* 'Top Crop') harvested 12–14 d after inoculation. Purification attempts with diseased tissue collected 19 d after inoculation yielded about 20% as much virus as was obtained at 14 d. The purification procedure (see Fig. 1) effected a complete separation between the virus and phytoferritin, which was present in copious amounts in extracts of GYMV-infected plants. Partially purified virus subjected to rate-zonal centrifugation in linear sucrose density gradients sedimented as a single band (Fig. 1).

The virus collected from density gradients was concentrated by ultracentrifugation and resuspended in 0.1 M sodium phosphate, *p*H 7.8, for analytical ultracentrifugation. Sedimentation velocity experiments showed a single sedimenting boundary (Fig. 2) with a mean sedimentation coefficient[7] ($S_{20,w}$) of 69.4±1.15. Attempts to determine the buoyant density of the intact virus in caesium chloride failed because the virus was disrupted by the salt.

Nucleic acid from GYMV was prepared by dissociating virus with 20 mM Tris-HCl, 5 mM sodium EDTA, *p*H 9.0, containing 2% (w/v) sodium dodecyl sulphate at 20° for 30 min (ref. 8). Dodecyl sulphate was precipitated by adjusting the mixture to 0.04 M KCl and storing it overnight at 4°. Nucleic acid was recovered from the supernatant liquid after low-speed centrifugation and dialysed overnight against 15 mM sodium citrate, 0.15 M sodium chloride (SSC), *p*H 7.0. Infectivity was tested by adding a small amount of carborundum to the nucleic acid in SSC and rubbing the mixture on expanding primary leaves of Top Crop beans with a sterilised ground glass spatula. Five days after inoculation typical symptoms of golden yellow mosaic appeared. Plants so inoculated were frozen and homogenised as before, and virus particles with the properties of GYMV (ref. 5) were obtained.

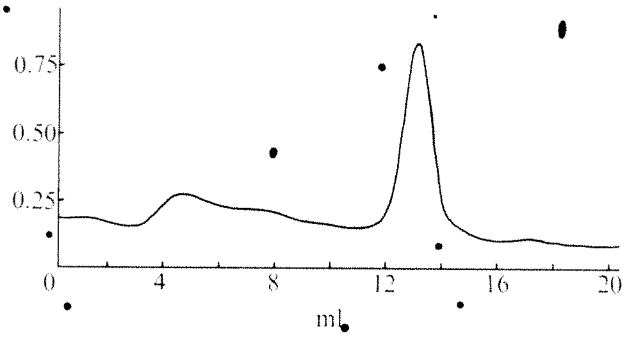

Fig. 1 Rate-zonal sedimentation profile of purified GYMV in linear 10–40% sucrose gradients. Leaves from infected plants harvested 12–14 d after inoculation were frozen on dry ice, crushed to a coarse powder and homogenised at 4 °C in 0.1 M sodium phosphate, 10 mM EDTA, 1 mM cysteine, *p*H 7.8 (3 ml buffer per g tissue). The homogenate was strained, clarified by centrifugation, adjusted to 0.2 M sodium chloride, and 4 g polyethylene glycol (Carbowax 6000, Union Carbide) was added per 100 ml clarified extract. The polyethylene glycol precipitate was collected by centrifugation after stirring for 2 h at 4 °C, and the virus dissolved from the precipitate in a small volume of 0.1 M sodium phosphate, *p*H 7.8. The virus was further purified by two cycles of differential centrifugation (12,000 r.p.m., 20 min, Sorvall SS-34 rotor followed by 27,000 r.p.m., 3.5 h, Beckman type 30 rotor) and then by rate-zonal centrifugation on linear sucrose density gradients (25,000 r.p.m., 4 h, Beckman SW 27.1 rotor). The sedimentation profile shown was obtained by scanning the sucrose density gradient from the top with an ISCO density gradient fractionator coupled to a Model UA-2 ultraviolet analyser.

The nucleic acid of GYMV sedimented in rate-zonal sucrose density gradients and the analytical ultracentrifuge as a single component (approximately 16S) but did not enter 2.5% polyacrylamide gels. Diphenylamine[9] and orcinol[10] tests were both positive, indicating that the nucleic acid of GYMV might be DNA. This interpretation was confirmed by nuclease sensitivity tests. Nucleic acid treated with bovine pancreatic DNase I (Worthington) was hydrolysed to oligonucleotides, whereas the nucleic acid was unaffected by treatment with RNase A (Sigma) (Fig. 3). The nucleic acid was unaffected by incubation for 4 h at 37 °C with 0.3 N sodium hydroxide, a treatment that hydrolyses RNA.

Only four other plant pathogenic viruses have been reported to contain DNA, and GYMV differs from each of these in several important respects. Cauliflower mosaic[11], dahlia mosaic[12] and carnation etch ring viruses[13] are aphid transmitted and have icosahedral particles of approximately 45 nm diameter. Cauliflower mosaic virus contains double-stranded DNA with a small amount of RNA covalently attached[14]. Potato leaf roll virus, which recent evidence suggests may

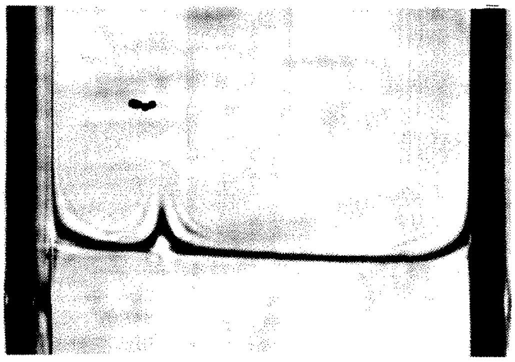

Fig. 2 Analytical ultracentrifuge Schlieren pattern of GYMV. Virus was adjusted to 0.5 absorption units (260 nm) in 0.1 M sodium phosphate, *p*H 7.8, and centrifuged in an An-D rotor at 35,660 r.p.m. Sedimentation is from the left. Photograph was taken 8 min after rotor reached full speed.

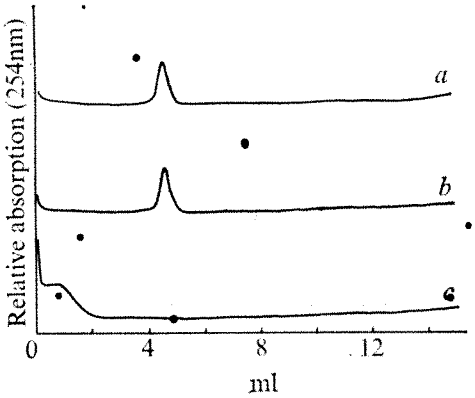

Fig. 3 Rate-zonal sedimentation profiles of GYMV nucleic acid in 10–40% linear sucrose density gradients following various treatments. Nucleic acid prepared in SSC, *p*H 7.0, as described in the text was adjusted to *p*H 5.0 and 5 mM Mg^{2+} by addition of 0.1 M sodium acetate, 50 mM magnesium sulphate, *p*H 5.0. Nucleic acid concentrations were adjusted to 10 μg ml^{-1}. To 0.5 ml portions were added 1 μl of *a*, water, *b*, RNase A (2.0 mg enzyme per ml), or *c*, DNase I (0.6 mg enzyme per ml water). Mixtures were incubated 4 h at 37 °C and then layered on sucrose gradients in 9/16 × 4 inch cellulose nitrate tubes and ultracentrifuged for 16 h at 25,000 r.p.m. in a Beckman SW 27.1 rotor. Profiles were obtained as in Fig. 1.

contain double-stranded DNA[15], is also aphid transmitted, and has particles 24 nm in diameter.

GYMV, the only whitefly-transmitted virus so far purified in sufficient quantity for characterisation, is superficially similar to several other viruses or virus-like particles of plants or fungi[16–18]. But there are as yet no reports on the nature of the genome of any of these particles. In size and nucleic acid type, GYMV most closely resembles the parvoviruses, small icosahedral viruses of animals that contain a single-stranded DNA genome[19–21]. Preliminary studies (unpublished) suggest that the DNA of GYMV may also be single-stranded. Thus GYMV and perhaps other whitefly-transmitted viruses may represent an entirely new class of plant pathogenic viruses.

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Biochemical characterisation of a serum thymic factor

THE search for biologically active peptides with hormonal properties has been intense. The thymus has been looked upon as a source of such materials by a number of investigators but so far no generally accepted compounds have emerged[1–5]. We present here a sequence for a nonapeptide which is found in the serum of several animal species, where its presence is thymus-dependent[6–8].

For some years we have been attempting to isolate and characterise this material, which we call FTS (*facteur thymique serique*) from the serum of various mammalian species[6–8] in particular the pig[8]; the procedure for extraction has been described in detail elsewhere[9]. Briefly, it consists of dialysis and Sephadex column purification, the stages of which are associated with a progressive increase in biological activity. This has been monitored chiefly by quantification of the effect of the extract on a rosette assay. There is a general correlation between results obtained with this method and with more obviously immunological restoration procedures (refs 9–13). The rosette assay is quick, reproducible[14,15] and amenable to a large-scale testing programme in spite of its somewhat esoteric nature.

Defibrinated pig blood (2,000 l) was ultrafiltered through polyacrylonitrile membranes, concentrated on an Amicon UM2 membrane and subjected to gel filtration on Sephadex G-25 (0.2 M phosphate buffer, *p*H 7.3). The active fractions were chromatographed on carboxymethyl cellulose (CMC) (0.02 M phosphate buffer, *p*H 6.3) and eluted with a NaCl gradient in a single peak at 0.12 M NaCl (Figs 1 and 2). Active fractions (1:4 × 10^7) were lyophilised and subjected to gel filtration on Sephadex G-25 packed in 5% acetic acid, and then on Sephadex G-10 in distilled water. The assay of biological activity used at each stage of purification[10,16] is based on the capacity of thymic extracts to render spleen rosette-forming cells from adult thymectomised (Tx) mice sensitive to inhibition by anti-θ serum and azathioprine. The purity of FTS thus isolated was assessed by (1) the presence of a single spot in thin-layer chromatography (TLC) in five different systems; (2) reproducible stoichiometry of amino-acid composition: Asx, Ser_2, Glx_2, Lys, Gly_2 Ala; (3) the absence of detectable amounts of these amino acids in the control unhydrolysed sample. Sequence studies were performed by Edman's technique as modified by Gray[17], using dansylation for free amino group determination.

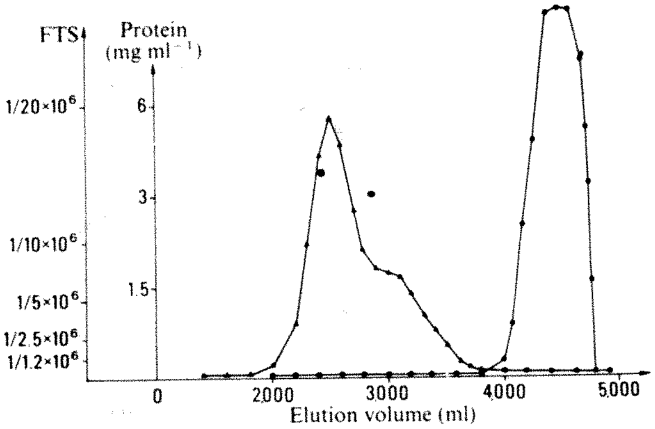

Fig. 1 Elution of pig serum ultrafiltrate on Sephadex G-25 (0.2 M phosphate buffer, *p*H 7.3). FTS activity was evaluated by the sheep cell rosette θ conversion assay. ▲, Proteins; ●, FTS.

As no NH_2 terminus could be identified in general, the sequence technique was applied to a tryptic digest with the following result: Ser–Glx–Gly–Gly–Ser. The COOH terminus, Asn, was evaluated, after treatment with carboxypeptides A, by dansylation. In one sample a faint glutamic acid spot was seen

Table 1 Principal biological activities of partially purified natural FTS

	Concentration dose	References
θ Conversion (Komuro and Boyse assay) *in vitro*	0.1 ng ml^{-1}	22
in vivo	15 ng per mouse	23
Enhancement of the generation of alloantigen reactive cytotoxic T-cells in Tx mice	15 ng weekly (per mouse)	13
Induction of suppressor T-cells in NZB mice assayed using antibody production against polyvinyl pyrrolidone	15 ng weekly (per mouse)	12
Enhancement of mitogen responses in nude mice (*in vitro*)	1 ng ml^{-1}	9
in adult Tx rats (*in vivo*)	75 ng (per rat)	24
Normalisation of the abnormally high level of autologous erythrocyte binding cells in adult Tx mice	15 ng (per mouse)	11

FTS was isolated on the basis of its action on the sheep cell rosette assay[8].

as the NH_2 terminal residue after dansylation; Edman degradation successively revealed Ala and Lys. The only amino-acid sequence compatible with all data reported above is:

Glx–Ala–Lys–Ser–Glx–Gly–Gly–Ser–Asn.

The amino group of residues 1 and 5 is still uncertain. It is likely, because of the difficulty of identifying free amino groups, that most of the NH_2 terminal residues are in the pyroglutamic form at the end of the isolation procedure (whether natively pyroglutamic acid or transformed *in vitro*). Such pyroglutamic acid, however, is apparently biologically active, for the blocked peptide is active in the rosette assay (as indicated by studies of biological material and confirmed by the activity of the synthetic peptide). Residue 5 is probably glutamine rather than glutamic acid in view of the isoelectric point of the peptide (7.3).

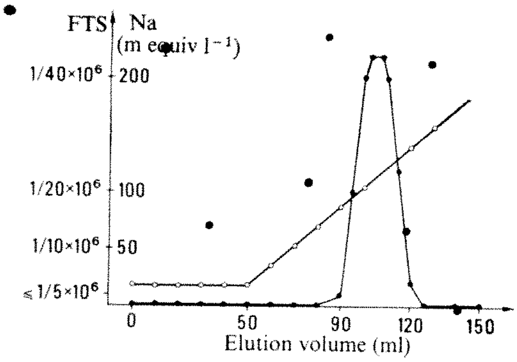

Fig. 2 Elution of Sephadex G-25 eluate after chromatography on CMC (0.02 M phosphate buffer, *p*H 6.3) and addition of NaCl. ●, FTS; ○, (Na+).

A peptide has been synthesised based on our sequence (with pyroglutamic acid as the NH_2 terminal residue) by the Merck peptide group (West Point, Pennsylvania) using Merrifield's technique of solid phase synthesis[18]. The synthetic peptide has been shown to behave like FTS on TLC (five systems) and had the same elution volume on Sephadex G-25 (0.2 M phosphate buffer, *p*H 7.3 and 5% acetic acid). Its biological activity was extremely high in the sheep cell rosette assay both *in vitro* and *in vivo*—of the same order of magnitude as natural FTS (Table 2).

In conclusion, we have presented direct evidence for the presence in normal blood of a thymus-dependent nonapeptide of molecular weight about 900, which adds to the list of biologically active peptides which could be hormones. The high activity of the synthetic peptide in the rosette assay of the peptide synthesised on the basis of the amino-acid sequence supports the specificity of its action. Its activity in various systems suggests that the natural peptide is involved in T-cell differentiation. These (Table 1) include (1) θ conversion *in vitro*[22] and *in vivo*[23]; (2) enhancement of the generation of alloantigen reactive cytotoxic T-cells in Tx mice[13]; (3) induction of suppressor T-cells in NZB mice assayed using antibody production against polyvinyl pyrrolidone[12]; (4) enhancement of mitogen response in nude mice *in vitro*[9] and ATx rats *in vivo*[24], and (5) normalisation of the abnormally high level of autologous erythrocyte-binding cells in ATx mice[11]. But the mechanism of such possible FTS involvement in T-cell differentiation is unclear. Most of the biological activities have been demonstrated in adult Tx and NZB mice which suggests that FTS effects the late stages of T-cell maturation. Too little is known to implicate FTS in the first stages of differentiation. It is possible that direct contact between T-cell precursors and thymic epithelium is then necessary whether or not it is mediated by humoral mediators. Alternatively, FTS may not be directly involved in T-cell differentiation in normal conditions and would be classified with previously described pharmacologically immunoactive peptides[19–20] which act in other systems. In any case, FTS might prove useful for the manipulation of T-cell subsets *in vivo*, particularly in clinical conditions showing subtle T-cell deficiency, probably involving the last stages of T-cell maturation, as is well documented in the spontaneously autoimmune NZB mice[21].

Table 2 Comparison of biological activities of natural and synthetic FTS

Sheep cell rosette θ conversion assay[8]	Natural FTS	Synthetic FTS
in vitro	$1–5\times10^{-2}$ pg ml^{-1} ($1–5\times10^{-14}$ M)	0.7×10^{-2} pg ml^{-1} (0.7×10^{-14} M)
in vivo	0.1 ng	0.1 ng
Autologous erythrocyte-binding cells[11]	0.2 ng	0.1 ng

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Lymphocytes suppressing both immunoglobulin production and erythroid differentiation in hypogammaglobulinaemia

CELLS blocking immunoglobulin (Ig) synthesis *in vitro* have been found among gradient-isolated blood mononuclear cells of individuals with variable immunodeficiency[1–4], immunodeficiency with thymona[1,3,4], infantile X-linked immunodeficiency[4], multiple myeloma[5] and Hodgkin's disease[6]. It has been suggested that the suppressor cells block terminal differentiation of human B lymphocytes[7]. The association of red cell aplasia and immunodeficiency states is well documented[8]. A circulating Ig capable of interfering with red cell formation has been demonstrated in several persons[9,10]. The majority of individuals with these disorders, however, have no circulating inhibitor of erythropoiesis. In the present study, lymphocytes from two persons with immunodeficiency with thymoma, one of whom had repeated episodes of red cell agenesis, were cocultivated in assay systems in which human blood B lymphocytes and human bone marrow erythroid precursors differentiate *in vitro*. The results suggest that the 'suppressor' cells observed in certain immunodeficiency diseases may act on both lymphocytic and haematopoietic target cells.

Two patients (S1 and S2) diagnosed as having immunodeficiency with thymoma were studied. Ig values for S1 were: IgG, 180; IgM, 0; IgA, 15 mg%; for S2: IgG, 305; IgM, 23; IgA, 22 mg%. Both individuals have had spindle cell tumours of the thymus removed and are currently receiving intramuscular gammaglobulin. Patient S1 has had three episodes of red cell agenesis documented by a falling haematocrit, absent blood reticulocytes, and absent bone marrow erythroid precursors. Remission was induced with steroids which have been tapered to 5 mg prednisone daily. Patient S2 is haematologically normal. In a previous report[11], patient S1, referred to as B.D. lacked most surface Igs from her blood lymphocytes, whereas S2 (M.P.) was in the normal range.

Lymphocytes were isolated from sterile blood of patients S1, S2 and controls by defibrination over glass beads followed by centrifugation on a Ficoll–Hypaque gradient[12], and cultured on the same day. Phagocytic cells were removed by sedimentation over a plastic Petri dish for 60 min at 37 °C. T lymphocytes were isolated by rosette formation with sheep red cells as described previously[13].

Measurement of Ig synthesis *in vitro* has been described previously[3,14] (for experimental details, see Table 1). Erythropoiesis *in vitro* was studied by determining the effect of the patient's serum and mononuclear cells on erythropoiesis using the number of erythroid colonies formed by normal human erythroid precursor cells in response to erythropoietin (EP) in the plasma clot system[15] (for details, see Table 2).

Cultured S1 and S2 mononuclear cells produced much less Ig *in vitro* than cells of normals in similar conditions. In single cultivation experiments shown on the top three lines of Table 1, the means for Ig production of 19 experiments on 14 normal controls were compared with combined results of experiments with S1 and S2. Individual experiments, from which the data in Table 1 were derived, generally included one patient and 1–3 controls tested on the same day. In such individual experiments there was never overlap between the patient and controls; S1 or S2 always produced 30% or less Ig than controls.

Table 1 Effect of blood mononuclear cells on Ig production of PWM-activated human lymphocytes *in vitro*

	Lymphocyte donor†	No. of expts‡	Ig (k+λ) S§	P§
			c.p.m. (mean ± s.e.m.)	
Single cultivation*				
	N	19	236±29	337±31
	S1	7	6±2	13±5
	S2	4	10±5	40±24
Co-cultivation*				
	S1+N	14	23±8	53±10
	S1+N(ADH)‖	2	37	42
	S1(T)¶+N	2	43	10
	S2+N	3	47±12	129±111
	N+N	4	261	402

* In single cultivation experiments 4×10^6 isolated mononuclears were cultured for 7 d at a concentration of 2×10^6 cells ml^{-1} in RPMI 1640 containing 17% foetal calf serum, 1% PWM (Grand Island Biol. Co., Grand Island, New York); and penicillin, streptomycin, and glutamine as described[3,13]. At the end of the culture period the cells were washed, suspended in leucine-free MEM and permitted to incorporate ^{3}H-leucine (10 µCi per 10^6 cells) for 4 h. Ig was determined on Triton-X-solubilised cell pellets and spent culture fluid fractions by a two-stage antibody immunoprecipitation procedure[3]. The mixed antisera had specificity for both kappa and lambda light chains. Data expressed as c.p.m. per 10^6 lymphocytes are after subtraction of control tube. Only experiments in which the control was <35% of the experimental results were considered valid. Cocultivation experiments were carried out with 4×10^6 cells of each cocultivant at 2×10^6 cells ml^{-1}. Preliminary cocultivation experiments showed no difference in Ig production when the cell concentration was varied from 1 to 4×10^6 cells ml^{-1}.

† N refers to normal control donors of lymphocytes. S1 and S2 are patients having immunodeficiency with thymoma.

‡ In the single cultivation experiments 14 different normals were involved in the 19 experiments. In co-cultivation experiments S1+N involved 6 different combinations of co-cultivants: S2+N involved 3 different combinations and N+N involved 4 combinations.

§ S refers to the secreted Ig found in the culture fluid after radio-incorporation while P refers to the cell fraction.

‖ Adherent cells were removed by leaving isolated mononuclear cells in a shallow plastic Petri dish for 60 min at 37 °C, decanting the cells which remain suspended, and using them. Latex ingestion indicated less than 2% of the remaining cells were macrophages.

¶ S1(T) were T lymphocytes isolated by the sheep red cell rosette method.

PWM is pokeweed mitogen.

Cocultivation experiments between S1 and S2 and normals demonstrated that both S1 and S2 blocked Ig production by cultured normal B lymphocytes. If the data are calculated as Ig c.p.m. as a percentage observed/expected (expected = 1/2 c.p.m. of normal + 1/2 c.p.m. of S1 or S2 in single cultivation), S1 is <20%; S2 is <40%. When normals were cocultivated together the observed/expected Ig production was close to 100% of the observed/expected.

Results for secreted and cellular Ig fractions in Table 1 were comparable, suggesting that the defect was not a failure to secrete but rather an inability to synthesise Ig. S1 quantitatively had greater suppressor activity than S2 in single cultivation and cocultivation studies.

Table 2 shows data for the plasma clot erythroid colony testing. In the absence of EP (lines 1–4 of Table 2) no erythroid colonies were formed from bone marrow or lymphoid elements. When EP was added, as seen in line five of Table 2, several hundred colonies were observed. Serum from S1 or S2 did not significantly alter the number of erythroid colonies observed (data not shown). Mononuclear cells from normal subjects enhanced the number of erythroid colonies: the differences noted were significant: the figure in line 6 is the mean of 28 experiments. When 1×10^5 lymphocytes from the putative suppressor cells, S1 and S2,

Table 2 Effect of mononuclear cells on erythroid colony formation by normal human bone marrow *in vitro**

Expt	Additions to culture	Number of colonies per 6×10^5 cells†	
1	None	0	
2	Normal BMC	0	
3	S1 BMC	0	
4	S2 BMC	0	
5	EP	328 ± 27	
			$P < 0.005$
6	EP+normal BMC	449 ± 32	
7	EP+S1 BMC	4 ± 2	
8	EP+S2 BMC	98 ± 21	
9	EP+S1 BMC+HC($10M^{-5}$)‡	16 ± 3	
10	EP+S2 BMC+HC($10M^{-5}$)	84 ± 10	
11	EP+S1 (Adh)§ BMC	—	
12	EP+S1(T)‖ BMC	10	
13	EP+S2 (Adh)§ BMC	—	

*1×10^5 lymphocytes were used.

† Each value represents mean ±1 s.e. of 3 separate studies each with 8 individual determinations.

‡ Hydrocortisone.

§ Adherent cells were removed by leaving isolated mononuclear cells in a shallow plastic Petri dish for 60 min at 37 °C, decanting the cells which remained suspended and using them.

‖ T lymphocytes isolated by sheep red cell rosette method[13].

BMC, Blood monocuclear cells.

Adh, Adherent.

Bone marrow cells obtained from haematologically normal individuals by aspiration from the posterior superior iliac crest were cultured in the presence or absence of 2 IU EP as follows. Each marrow (6×10^5 cells) was cultured with media containing either normal human or patient serum (0.05, 9.1 and 0.2 ml per culture), normal human lymphocytes or patient's lymphocytes (0.5, 1, 2, 4 and 6×10^5 lymphocytes per 6×10^5 marrow cells). The cultures were carried out for 5–7 d at 37 °C in a humidified atmosphere of 5% CO_2 in air. Only colonies composed of 8 or more erythroid cells were considered in the tabulation of the results.

were added, there was significant decrease in the number of colonies. The degree of suppression is greater for S1 than for S2. Hydrocortisone (lines 9 and 10, Table 2) did not affect the suppression phenomenon.

When adherent cells were removed from S1 and S2 mononuclear cell populations (Tables 1 and 2) there was no loss of suppression in either the Ig production or plasma clot system. Isolated T cells could suppress cocultivated cells in both systems, suggesting that the suppressor cell(s) was a T lymphocyte.

The data demonstrate that lymphoid cells which interfere with the maturation of both human erythroid precursors and of B lymphocytes in culture are present in the blood of two persons with immunodeficiency with thymoma. By contrast, cells from healthy controls stimulate the production of erythrocytes in the plasma clot system and leave Ig production essentially unaffected in the PWM-activated Ig production assay.

T lymphocytes and adherent cells, which suppress Ig production *in vitro*, are present in a number of immunodeficiency diseases[1-7]. Caution must be exercised in interpreting these cells as aetiologic in view of recent data which show that suppressor T lymphocytes are part of the normal immunoregulatory elements in the immune response[16]; suppressor cells are induced by lectins and other agents[17]; and suppressor cells are found in the genetically determined disorder, infantile X-linked immunodeficiency[4], in which an inherited defect determining suppressor cells would be less likely than the presence of such cells as an epiphenomenon. In the present study, the observations that S1 possessed suppressor cells for erythroid cell development while in remission from her anaemia, and S2 had similar cells although haematologically normal, make it unlikely that these cells were the sole cause of the anaemia in S1.

Recent studies have focused on the role of 'suppressor' mononuclear cells in haematological disorders—particularly disorders in which there is arrest of bone marrow cells. Suppressor cells of erythropoiesis have been found in cases of Diamond–Blackfan syndrome using the plasma clot assay[18]. There are recent observations implicating blood mononuclear cells in selected cases of aplastic anaemia[19,20]. The present paper, however, is the first in which the same patient has been shown to have suppressor cells for both lymphoid and erythropoietic cells.

The lack of change in suppression after removal of adherent cells and its production by T cells is consistent with the suppressor cells being T lymphocytes in both *in vitro* assays. It is not known whether separate cell subpopulations are involved. Dissociation of suppression of Ig production from suppression of erythroid cell maturation has, however, been noted in cases of Blackfan–Diamond syndrome[18], and in primary immunodeficiency.

The difficulties in evaluating the relationship between 'suppressor' cells and disease states is complicated by the different types of cells which may possess suppressor activity, and the differences between *in vitro* and *in vivo* conditions. Nevertheless, the present data raise the possibility that suppressor lymphocytes play a role in the pathophysiology of diseases in which both lymphocytic and hematopoietic abnormalities coexist.

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Reduced rates of proteolysis in transformed cells

GROWTH of cells implies a net accumulation of intracellular proteins with the rate of protein synthesis exceeding the rate of protein degradation. It follows that the seemingly uncontrolled growth of transformed cells could be produced by a reduction in intracellular proteolysis without any concomitant increase in protein synthesis. In order to examine this hypothesis we have determined the rates of protein degradation in two pairs of (non-isogenic) cell types—non-growing rat liver hepatocytes and the chemically transformed Reuber H35 hepatoma, and mouse BALB/c 3T3 fibroblasts and 3T3 fibroblasts transformed with Simian Virus 40 (SV40). We have measured proteolysis over a wide range of insulin concentrations with cells incubated in either an enriched medium or a basal salts medium (step-down conditions). Insulin was chosen because it inhibits intracellular proteolysis in several cell types[1-6] and may have a major role in the normal regulation of proteolysis *in vivo*. We report here that transformed cells have a lower basal rate of proteolysis together with an increased sensitivity towards inhibition by insulin, two factors which will contribute to the rapid growth of such cells.

Cell proteins were labelled by a 16-h incubation with ^{3}H-leucine and protein degradation was followed after an intervening 3-h chase (Fig. 1). This chase period is desirable for the effective removal of unincorporated ^{3}H-leucine and essential to allow for the insulin-insensitive degradation of short-lived proteins[4,7]. Leucine (2 mM) was added during both the chase and degradation periods to reduce the specific activity of ^{3}H-leucine released from labelled proteins and thus suppress any reincorporation of isotope which would cause an apparent reduction in proteolysis.

We found substantial differences between hepatocytes and hepatoma cells in the overall rate and regulation of intracellular proteolysis (Fig. 1). Breakdown of 14% of the labelled hepatocyte proteins occurred during a 4-h incubation in salts medium. This rate is 50% greater than found for Reuber hepatoma cells labelled and incubated in identical conditions Furthermore, the lower rate of protein degradation in hepatoma cells also occurred when the experiment was performed in Eagle's Minimal Essential Medium (MEM)[9]. Insulin addition at the beginning of the degradation period inhibited intracellular protein degradation in both cell types, but was effective at much lower concentrations in the hepatoma. Indeed the insulin concentration for half maximum inhibition in Reuber hepatoma cells was approximately 10^{-12} M, over two orders of magnitude less than the concentration required for comparable inhibition of proteolysis in hepatocytes (Table 1). Moreover, across the whole range of insulin concentrations tested, and even at concentrations producing maximum inhibition of proteolysis, rates of protein degradation in the hepatoma cells were still half those found for hepatocytes.

The lower proteolytic rate and higher sensitivity of protein degradation to insulin in hepatoma cells may reflect a difference between growing and non-growing cells rather than a difference between transformed and normal cells. The omission of serum during the 3-h chase and 4-h degradation periods, however, would restrict growth of the hepatoma line, especially when incubated in a simple salts medium without amino acids. It seems more likely therefore that the differences in proteolytic rate between the two cell types reflect a changed genotype independent of cell growth rates.

This conclusion is supported by data obtained with mouse 3T3 and SV40-3T3 fibroblasts which differ by the insertion of viral genome[12,13]. Proteolysis measurements on these cell types show qualitatively similar results (Fig 2) to those described for hepatoma cells and hepatocytes. Thus control rates of protein degradation in nutritional step-down

Table 1 Insulin concentrations for half maximum inhibition of protein degradation

Cell type	Medium	
	Salts	MEM
Rat hepatocytes	7×10^{-10} M	1×10^{-9} M
Reuber H35 rat hepatoma	3×10^{-12} M	2×10^{-12} M
3T3 (BALB/c3T3) mouse fibroblasts	3×10^{-9} M	1×10^{-9} M
SV40 transformed 3T3	2×10^{-10} M	6×10^{-11} M

Values were calculated from the data in Figs 1 and 2.

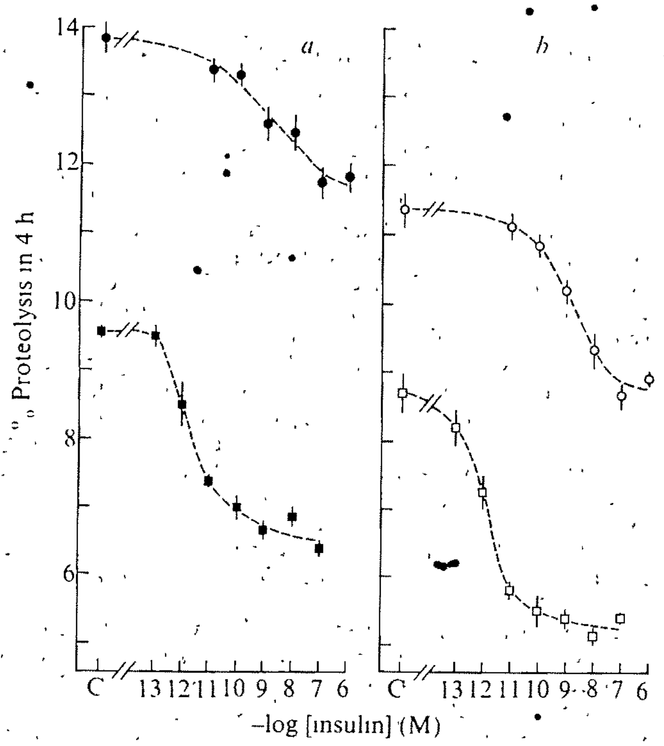

Fig. 1 Proteolysis in hepatocytes and Reuber H35 hepatoma cells. Livers from fed, 150-g rats were perfused with medium containing collagenase to disperse the tissue[8]. Cells were isolated by shaking the dispersed tissue in perfusion medium at 37 °C for 15 min, and filtration through nylon gauze. The cells were pelleted by centrifuging at 50*g* for 1 min, suspended in calcium-free Eagle's MEM[9] containing 25 mg gentamycin per 100 ml, and transferred to sterile centrifuge tubes. Aseptic techniques were then adopted for subsequent preparation and incubation steps with the isolated cells. The cell suspension was centrifuged as before, the supernatant discarded, and the cell pellet washed once in the same buffer A final suspension of approximately 10^8 parenchymal cells was transferred to 250 ml of MEM containing 20 mM N-Tris [hydroxymethyl]methyl-2-aminoethane sulphonic acid (TES) *p*H 7 5, 25 mg gentamycin, 25 mg streptomycin sulphate, 15 mg penicillin G, 2 5 mg fungizone and enriched with amino acids and vitamins at twice the recommended amount[9], together with 10% foetal calf serum (enriched MEM). Aliquots of 5 ml of this diluted cell suspension were dispensed into 60-mm plastic Petri dishes and transferred to a humidified incubator at 36.5 °C and gassed with 5% CO_2 in air Almost all the hepatocytes attached to the dishes and after 24 h the cells showed a marked morphological change from birefringent spherical cells to translucent flattened cells with clearly visible intercellular abutments. At this time the medium was changed to one with similar constituents but containing ^{3}H-leucine 1 μCi ml^{-1} (specific activity 50 Ci mmol^{-1}) and with non-radioactive leucine omitted. The cells were incubated in this medium for a further 16 h to label intracellular proteins The medium was then changed to MEM containing 20 mM TES and 2 mM leucine but without serum (chase MEM). After a further 3-h incubation the plated cells were washed twice with Earle's salts and incubated either in *a*, Earle's salts with 20 mM TES and 2 mM leucine added (chase salts) or in *b*, chase MEM Insulin (Actrapid, Novo) was added at the concentrations indicated. After 4 h the cells were collected in medium by scraping with a rubber policeman after addition of Triton X100 to a final concentration of 0.5%, then thoroughly mixed on a vortex mixer and protein was precipitated from 250-μl portions by the addition of trichloroacetic acid (TCA) to a final con centration of 20%. The TCA mixtures were kept for 1 h at 0 °C, centrifuged for 10 min at 2000*g* and the radioactivity in both TCA-soluble and TCA-insoluble fractions measured using NCS Solubilizer (Searle Nucleonics) in a toluene scintillation solution[10]. Proteolysis was expressed as the percentage of total radioactivity in the TCA-soluble fraction (amino acids) after subtracting the TCA-soluble counts at zero time. Reuber H35 hepatoma cells[11], were grown to a similar density to that obtained with hepatocytes. Subsequent procedures were as for hepatocytes. Values shown for hepatocytes (●, ○) and hepatoma cells (□, ■) are means ± s.e m. for data on between 6 and 10 dishes at each insulin concentration and for control incubations (C) in the absence of insulin.

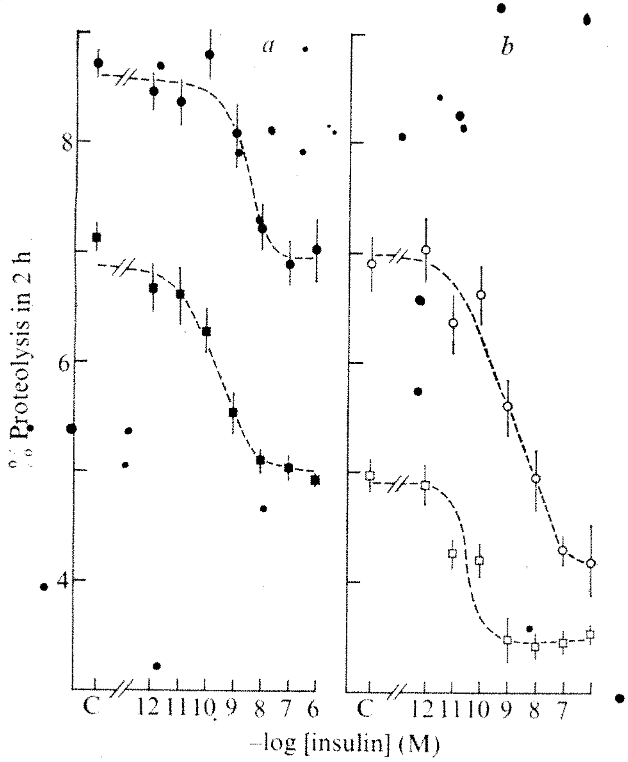

Fig. 2. Proteolysis in BALB/c 3T3 and SV40-3T3 cells. The cells were grown in enriched MEM. Labelling, chase conditions and washing were as described in Fig. 1, except for the omission of foetal calf serum during the labelling period. Collecting and radioactivity measurements in cell protein, medium protein, cell amino acids and medium amino acids were as described before[5,10]. Proteolysis is expressed as the percentage of total radioactivity in the sum of the two amino acid fractions. Values shown for 3T3 cells (○, ●) and SV40 3T3 cells (□, ■) are means ± s.e.m. for data on 6–15 dishes at each insulin concentration and for control incubations (C) in the absence of insulin. *a*, incubation in chase salts; *b*, incubation in chase MEM.

conditions (salts) are 25% higher in 3T3 compared with SV40-3T3 cells. A slightly greater difference is found when the comparison is made between the two cell types incubated in MEM. Furthermore, proteolysis is more sensitve to inhibition by insulin in the transformed fibroblast than in 3T3 cells. Such differences (Table 1) are less than seen between hepatoma and hepatocytes, but there is still a 15-fold difference in the concentration of insulin required to inhibit protein degradation by 50%.

Lower basal rats of protein degeradation and higher sensitivity of the process to insulin inhibition will be important contributing factors towards rapid protein accumulation and growth of cancer cells. The lower basal rate of protein degradation in transformed cells might represent a partially inhibited condition. This is unlikely, however, since the extent of insulin inhibition in transformed cells is at least as great as found for normal cells. Rather, the difference in insulin sensitivity might be due to greater receptor affinity on the plasma membrane of transformed cells. Alternatively, a higher rate of insulin degradation in the non-cancer cells might account for the higher concentration of insulin required to inhibit protein degradation. But, this would occur only if the receptor affinity in the non-cancer cells was great enough to account for the half-maximal effects on degradation in the cancer cells. This does not seem to be the case since the apparent K_D for insulin binding in both hepatocytes[14] and fibroblasts[15] is lower (10^{-9} M) than needed to satisfy this restriction, and in good agreement with our calculation of the half-maximum effect of insulin on proteolysis in normal cells. Therefore we consider it most likely that transformed cells have a higher receptor affinity for insulin and that this largely determines their lower rates of protein degradation, although the mechanisms involved remain obscure.

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Thermogenic defect in pre-obese *ob*/*ob* mice

YOUNG genetically obese (*ob*/*ob*) mice develop obesity even when pair-fed to their lean littermates[1–3]. This is due to an unusually high metabolic efficiency, which we have suggested results from a reduction in the "maintenance increment" to the basal metabolic rate (BMR)[4]. Factors involved include the thermic effect of food and the thermogenic response to an environmental temperature below thermoneutrality. Part of the evidence supporting this suggestion comes from the observation that mature *ob*/*ob* mice, and at least two other strains of genetically obese rodent, are unable to maintain their body temperature when exposed to cold[5–7]. At 4 °C both the *ob*/*ob* mouse and the Zucker (*fa*/*fa*) rat rapidly die of hypothermia[5,7]. In the mouse, thermogenesis rather than heat conservation has been established as the cause of the thermoregulatory defect[5,7]. We now report that a defective response to cold is apparent in preweanling mice bearing the *ob*/*ob* genotype about 2 weeks before they can be identified as obese by visual inspection. From this a test based on the response to 4 °C has been developed for the identification of *ob*/*ob* mice at 17 d old.

Exploratory studies showed that 17 d was the earliest age at which preweanling 'normal' mice can maintain their body temperature when placed in a cold room (4 °C) for up to 90 min. This age was therefore taken as the base-line for comparison of the response to 4 °C of lean and 'pre-obese' animals. Litters were derived from lean parents heterozygous for the *ob* gene (*ob*/−) since the obese (*ob*/*ob*) female is infertile and the obese (*ob*/*ob*) male is too corpulent to mate. Mice were placed at 4 °C for up to 60 min and their rectal temperature was measured periodically. Each litter consisted of two groups: those in the larger group, although showing some change, could maintain their body temperature within the normal range; those in the smaller group exhibited a marked decrease and had a lower initial temperature. Temperature response curves of mice from nine litters are shown in Fig 1. Mice unable to maintain a normal temperature were marked for subsequent identification and all members of each litter were returned to their nest. Each animal was then examined at 28–30 d old, the earliest age at which obese individuals can be identified by visual inspection. In all litters the mice that had been marked were obese and those that were unmarked were lean. Clearly, 17-d-old mice with the *ob/ob* genotype are less able than normal mice to respond to the cold.

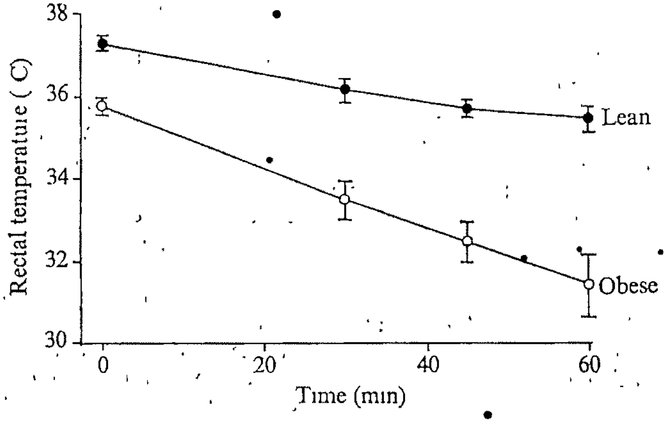

Fig. 1 Rectal temperatures of 17-d-old mice in response to an environmental temperature of 4 °C. Mice were removed from the nest and their initial temperature was taken by inserting a thermocouple (diameter <1 mm) 10 mm into the rectum; the thermocouple was attached to a Comark 3001 digital thermometer. Each mouse was individually caged and immediately transferred to a 4 °C room. Further temperature measurements were taken periodically. The results are expressed as the means ± s.e.m. for 39 lean animals (●), and for 16 animals that were subsequently found to carry the *ob/ob* genotype (○). At each time the temperature difference between the two groups was statistically significant, $P < 0.001$, on the basis of a Students' *t*-test.

More litters were then examined with the aim of using the impaired response to a 4 °C cold stress as a test for the *ob/ob* genotype. Such a test is essential if the metabolic changes preceding the development of obesity are to be elucidated. In total 133 mice from 19 litters were used. Before the experiment 7% were discarded as either abnormally small or in poor condition. The rest were categorised as 'definitely lean' or 'definitely obese' according to how they tolerated the cold. Some of those classified as definitely obese had such a precipitous fall in temperature that they had to be returned to the nest before the full 60-min test was completed. 19.5% of animals were predicted to be obese and 72.4% to be lean; the genotype of all these animals was found to be correctly predicted when they were examined for obesity at 28–30 d (Table 1). A few (8.1%) that could not be categorised readily were termed 'tentatively lean.' These showed a fall in temperature outside the normal range, but much less than for the 'definitely obese' mice. Of the 'tentatively lean' animals, two were found to be obese and eight to be lean. Clearly at 17 d-old, individuals that will be lean or obese can be obtained with certainty from litters derived from heterozygous parents. Genotype was predicted correctly for 98% of animals.

The stressfulness of the method of identification seems not to be a problem, for we have not observed an increased morbidity or mortality in cold-stressed mice, nor have we found long term effects on their growth rate and reproductive performance. Kaplan and Leveille[8] identified *ob/ob* mice between 18 and 20 d old by their reduced oxygen consumption at 25 °C. The main advantages of our procedure, apart from the marginal improvement in the age at which the genotype can be identified, are its simplicity and the ease with which many animals can be examined in a short time.

Table 1 Prediction of the genotype of lean and *ob/ob* mice aged 17 d by their response to an environmental temperature of 4 °C

Prediction		Result		%
Category	No	No. obese	No. lean	Correctly predicted
Definitely obese	24	24	0	100
Definitely lean	89	0	89	100
Tentatively lean	10	2	8	80

To investigate whether the thermoregulatory defect of *ob/ob* mice is detectable earlier than 17 d we examined the response to environmental temperatures less severe than 4 °C in mice aged between 12 and 17 d. Animals younger than 12 d could not be used because even our finest thermocouple probes caused rectal bleeding. With 12 d-old mice and an environmental temperature of between 15 and 20 °C *ob/ob* and lean animals were sharply differentiated. The former showed a marked fall in body temperature within 15 min of exposure to the cold (Fig. 2). The fact that defective thermoregulation occurs in *ob/ob* mice as young as 12 d indicates that a test similar to that for 17-d-old animals could be used for their identification.

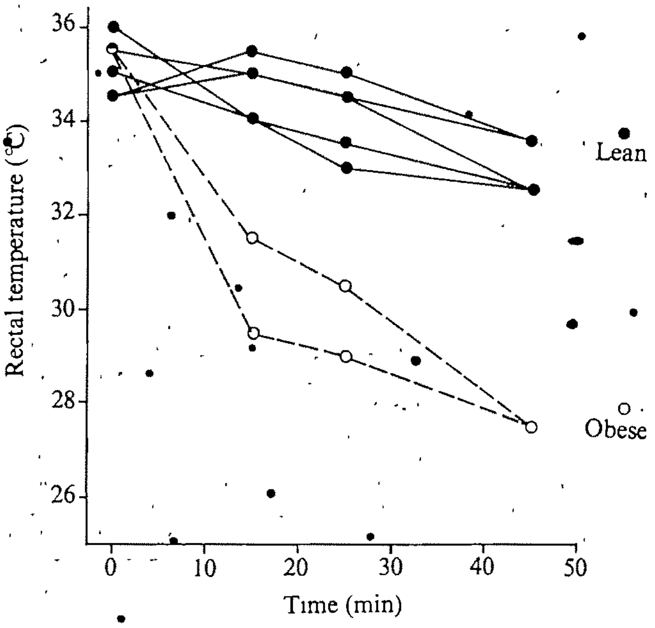

Fig. 2 Rectal temperatures of a litter of 12 d-old mice in response to an environmental temperature of 15 °C. Experimental details are the same as in Fig. 1. The animals fell into two groups, those subsequently found to be *ob/ob* (○) and those found to be lean (●).

The work reported here shows that the defective thermoregulation exhibited by mature *ob/ob* mice is not a secondary consequence of obesity. Because the defect in the adult animals is due to an impairment in the capacity for thermogenesis rather than in heat conservation[6,7], the same cause presumably applies to the defect in the pre-obese animals. These results therefore support the suggestion that a reduction in the "maintenance increment" of an animal's energy expenditure is a fundamental factor in the increased metabolic efficiency that leads to obesity[4]. In mice non-shivering thermogenesis is a major contributor to energy expenditure when the animals are maintained, as is usual, about 10 °C below their critical temperature. Thus, reduced non-shivering thermogenesis may account for the enhanced metabolic efficiency of *ob/ob* mice.

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Post-weaning food restriction reduces adipose cellularity

KNITTLE and Hirsch[1] reported that varying the calorific intake of newborn rats by manipulating litter size during the 21-d suckling period followed by allowing free access to food after weaning caused the rats from small litters to have more adipocytes in the epididymal depot than rats from large litters. Hirsch and Han[2] reported that marked changes in depot size induced by starvation or experimental obesity produced no change in total number of adipocytes in the epididymal and retroperitoneal depots of rats even when these alterations occurred as early as 6 weeks of age. These and other studies[3–5] suggest that it is the calorific intake between birth and weaning rather than later in life that plays the important role in establishing the cellularity of the adipose tissue depots of the adult. But we report here data which indicate that a reduction in food intake can markedly influence the number of adipocytes in the depots of adults even when initiated as late as 6 weeks of age.

Barrier-bred SPF Fisher 344 rats (28 d old) (Charles River) were kept in a barrier facility. All rats were initially fed Ralston-Purina diet 5755. Body weight and food consumption were recorded for 2 weeks after which the diet allotment for half the rats was reduced over 1 week to 60% that of '*ad libitum* rats'. Rats on the restricted food intake received diet 5755-4 (Ralston Purina) which was identical to 5755 except the vitamin content was increased so that vitamin consumption was the same in both groups of rats. Thus of the 20 rats killed at 6 months of age, 10 were fed *ad libitum* for their entire life and the other 10 were kept on the restricted food intake from 6 weeks to 6 months of age.

At 6 weeks of age, the 10 rats to be fed *ad libitum* throughout their life ('*ad libitum* rats') averaged 93±2.7 g (s.e.m.) and the 10 rats to be fed a restricted amount of food from that time on ('restricted rats') averaged 96±5.7 g. At 6 months of age the *ad libitum* rats averaged 344±8.1 g and the restricted rats 212±4.2 g.

Table 1 Left epididymal and perirenal adipose depot masses in *ad libitum* and restricted rats

Adipose depot	Total depot mass (g)*		Depot mass (g) per total body mass (kg)*	
	Ad libitum rats	Restricted rats	*Ad libitum* rats	Restricted rats
Epididymal	5.0±0.25	1.7±0.16†	14.6±0.74	7.9±0.74†
Perirenal	5.1±0.21	1.5±0.17†	14.7±0.61	7.3±0.77†

Rats were killed by decapitation and perirenal and epididymal fat depots were removed and placed in 0.9% NaCl at 37 °C. Adipocyte size and cell number per depot were determined by a method modified from Stiles *et al.*[9], using type IV collagenase (Worthington lot 45M181X, 190 units mg^{-1}). Crystal violet was used to stain the nuclei of adipocytes, collagenase, stromal material and the small, more slowly floating cells were removed from adipocytes by allowing the latter to float to the surface of 15 ml digestion medium without collagenase. The infranatant was discarded and the washing of the adipocytes was repeated twice. This procedure did not influence the adipocyte size distribution when compared to the procedure of Stiles *et al.* which does not remove these contaminating materials before sizing the adipocytes.

*Data are means of 10 depots ± s.e.m.

†Significantly different from *ad libitum*-fed rats ($P<0.001$)

The restricted rats had smaller fat pads than did their *ad libitum* counterparts (Table 1). Furthermore, expression of these data as mass of depot per kg body mass showed that adipose tissue depots of the restricted animals contributed less to body mass than depots of *ad libitum* rats.

The mean diameter of the adipocytes in the epididymal or perirenal adipose depots of the restricted rats was smaller than that of the adipocytes from the *ad libitum* rats (Table 2). The total number of adipocytes in the left epididymal and perirenal fat depots of the restricted rats was markedly lower than the number in the fat depots of the *ad libitum* rats.

The pattern of development of adipose tissue in rats is well established[2,7,8]. From the time of weaning until approximately 14 weeks of age, the epididymal, retroperitoneal and subcutaneous depots grow by increases in both adipocyte size and number. At about the age of 14 weeks to at least 6 months of age the cell number is nearly fixed[2,6,10]. Similar patterns of adipose tissue development have been observed in many but not all species studied[1,11,12].

Table 2 Adipocyte size and number of adipocytes in left epididymal and perirenal adipose depots

	Ad libitum rats	Restricted rats
Adipocyte diameter (μm)*		
Epididymal	107±2.4	84±3.0†
Perirenal	114±2.6	86±4.0†
Adipocytes (× 10^6) per depot*		
Epididymal	7.4±0.38	4.6±0.19†
Perirenal	6.1±0.27	3.9±0.17†

*Data are means of 10 depots ± s.e.m.

†Significantly different from *ad libitum*-fed rats ($P<0.001$)

Although the number of adipocytes in the depots increases well past weaning, it is generally held that the number found in depots of the adult rat can be influenced by modifying calorific intake only if initiated in the preweaning period[1,5]. On the basis of the studies with rats and on data obtained with man, it has been suggested that the time between birth and 2 yr of age is critical for the development of human adipose tissue and in the pathogenesis of human obesity[13]. of human obesity[13].

Although calorific restriction of adults has not been shown to influence the number of adipocytes in their depots, some nutritional manipulations in adults do influence adipocyte number. High-fat diets increase the number of adipocytes in the depots when fed to adult rats in the presence of adequate amounts of B vitamins[14–16].

Our results show that the number of adipocytes in the depots of adult rats can be markedly reduced by food restriction initiated 2 weeks after weaning. The preweaning period is thus not the only period sensitive to reduction in food intake in regard to establishing adipocyte number in adult depots. How much later in childhood this nutritional regime can be initiated and still have a sizeable effect on adipose tissue cellularity is of great interest, but the design of our ageing research programme precluded obtaining such data. Whether food restriction initiated at 6 weeks of age leads to permanent reduction in adipocyte number (that is, one not reversed by *ad libitum* feeding), as is the case with preweaning restriction, was also not explored. It is possible that control of the development of adipose tissue cellularity may well be in the province of the paediatrician's work with schoolage children.

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Proline analogue removes fibroblasts from cultured mixed cell populations

PRIMARY cultures of cells from various tissues have become important tools for many experimental studies, but attempts to prepare primary cultures of specialised cell types are frequently frustrated by the tendency of fibroblasts to grow more rapidly than other cells. During studies on procollagen biosynthesis by fibroblasts, it has been found that if the cells are incubated with any one of four proline analogues, the analogues are incorporated into protein and the incorporation of the analogues into procollagen polypeptides prevents the chains from folding into a triple helicial conformation[1-7]. We report here that if fibroblasts are grown in the presence of the proline analogue *cis*-hydroxyproline, their rate of growth is markedly limited. This may provide a simple technique for removing fibroblasts from cultures of mixed cell populations.

Fibroblasts isolated by controlled enzymatic digestion of the tendons of 17-d-old chick embryos, and primary cultures were prepared as described before[8]. Addition of 100 μg ml^{-1} of *cis*-hydroxyproline (*cis*-4-hydroxy-L-proline or allohydroxy-L-proline; Ajinomoto) to logphase cultures decreased the growth rate within 24 h (Fig. 1*a*) and thereafter the number of cells per flask decreased to less than the initial level. The same concentration of *cis*-hydroxyproline had no effect on the growth of KB cells (Fig. 1*b*), a cultured line of cells developed from a human epidermoid carcinoma. Also, it had no effect on the growth of a neuroblastoma cell line (Fig. 1*c*).

Examination of varying concentrations of *cis*-hydroxyproline (Fig. 2) demonstrated that the growth of primary cultures of tendon cells in late-log phase was inhibited with 50 μg ml^{-1} or more of *cis*-hydroxyproline. Cultures of mouse L-929 fibroblasts were more resistant and a concentration of 100-200 μg ml^{-1} was required to inhibit the growth. The growth of neuroblastoma or KB cells was not affected with 200 μg ml^{-1}, but there was partial inhibition with higher concentrations. The sensitivity to *cis*-hydroxyproline was not related in any simple manner to the rate of cell growth; in the experiments reported here the doubling time was 16 h for the primary cultures of tendon fibroblasts, 30 h for the L-929 fibroblasts, 24 h for the KB cells, and 24 h for the neuroblastoma cells (Fig. 1).

The selective effect of *cis*-hydroxyproline on fibroblasts was further illustrated by preparing mixed cultures of fibroblasts and KB cells, and then labelling the cultures with antibodies specific for prolyl hydroxylase[9], the enzyme which synthesises the hydroxyproline in procollagen. The antibody was conjugated with peroxidase and located in cells by reacting the peroxidase with 3,3′-diaminobenzidine[10] (Sigma). Figure 3*a* shows that fibroblasts were readily distinguished from KB cells in untreated cultures. In cultures treated with 100 μg ml^{-1} of *cis*-hydroxyproline for 7 d, KB cells were present but no fibroblasts were discernible either by morphology or a specific antibody stain for prolyl hydroxylase (Fig. 3*b*).

In a separate series of experiments, cultures in late log phase were incubated with ^{14}C-proline and then assayed for non-dialysable ^{14}C-hydroxyproline[8,11], as a measure of the rate of procollagen synthesis. Cultures which were the most sensitive to *cis*-hydroxyproline (Fig. 2) had the highest rate of procollagen synthesis (Table 1). Also, after mixed cultures of tendon fibroblasts and KB cells were grown in the presence of *cis*-hydroxyproline for 5 weeks, there was no apparent recovery of procollagen synthesis when the cells were subsequently subcultured without *cis*-hydroxyproline for 1 week. Morphologically the cells were similar to KB cells. These results suggest that the fibroblasts were effectively removed from the mixed cultures.

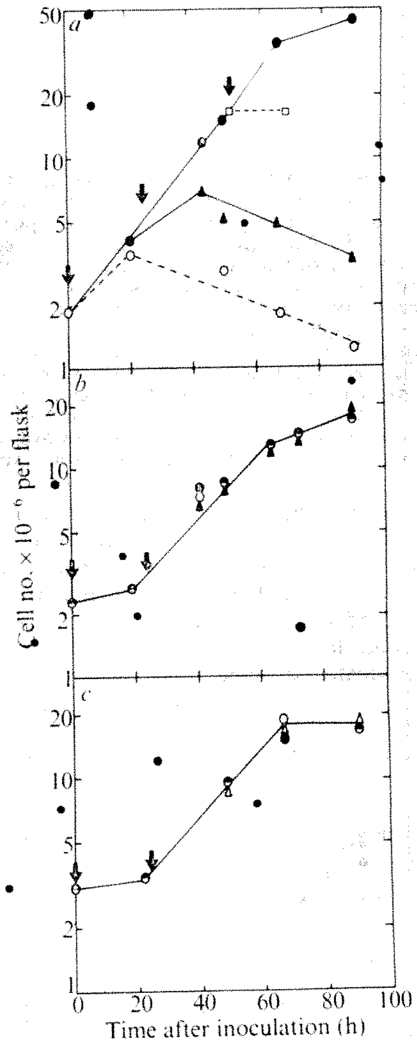

Fig. 1 Effects of *cis*-hydroxyproline on the growth of *a*, tendon fibroblasts; *b*, KB cells and *c*, neuroblastoma cells. Tendon fibroblasts were obtained from limited enzymatic digestion of tendon from 17-d-old chick embryos[8]. KB cells (CCL-17) and neuroblastoma cells (CCL-131) were from the American Type Culture Collection. The cells were grown in an atmosphere of 10% CO_2, and 90% air in Dulbecco's medium supplemented with 10% foetal calf serum, 100 units ml^{-1} of penicillin, and 100 μg ml^{-1} of streptomycin. The culture media were changed every other day after inoculation. *cis*-Hydroxyproline (100 μg ml^{-1}) was added to cultures at time indicated by arrow. Cell number per flask (Falcon; 75 cm^2) was estimated with a haemacytometer. Values presented are the mean from duplicate cultures. Cultures were grown in control conditions (●), or with *cis*-hydroxyproline added at time of inoculation (○), 24 h after inoculation (▲), or 54 h after inoculation (□).

The effect of *cis*-hydroxyproline on the growth of fibroblasts in culture might be explained by the previous observations indicating that when cells are incubated with proline

Table 1 Effect of *cis*-hydroxyproline on synthesis of non-dialysable ^{14}C-hydroxyproline by cell cultures

Cells	Treatment	^{14}C-hydroxyproline synthesised (d.p.m. per 10^7 cells per 30 min)
Tendon fibroblasts		5,691
L-929 fibroblasts		844
KB cells		< 100
Neuroblastoma		< 100
Tendon fibroblasts		4,974
Tendon fibroblasts + KB cells	+cHyp, 6 weeks*	< 100
Tendon fibroblasts + KB cells	+cHyp, 5 weeks; −cHyp, 1 week†	< 100

For labelling, medium was removed from each flask that contained $2-5\times10^7$ cells on the 6th day after the last subculturing and fresh medium (without *cis*-hydroxyproline) containing 1 μCi ml^{-1} of ^{14}C-proline was added (10 ml per flask) as described before[8]. In the second experiment, labelling period was 4 h instead of 30 min and values are reduced proportionally to make them comparable to the first experiment. Assay did not permit detection of less than 100 d.p.m. of ^{14}C-hydroxyproline.

* Mixed cultures (1:1) of tendon fibroblasts from chick embryos and KB cells were incubated continuously with 100 μg ml^{-1} of *cis*-hydroxyproline (cHyp) through 6 subcultures over 6 weeks.

† Mixed cultures (1:1) were incubated continuously with 100 μg ml^{-1} of *cis*-hydroxyproline through 5 subcultures over 5 weeks and then for a 6th subculture without the analogue for 1 week.

analogues, the analogues are incorporated into procollagen and the procollagen containing the analogues cannot become triple helical[1-7]. Several observations have suggested that the secretion and deposition of triple-helical collagen on surfaces used to culture cells may enhance the rate of growth of some fibroblasts *in vitro*[8-12]. It is possible therefore that *cis*-hydroxyproline limits the rate of fibroblast growth in the conditions employed here because it prevents the deposition of triple-helical collagen on the cell layer. This hypothesis, however, has not been explored in detail and it may well be that *cis*-hydroxyproline decreases the growth of fibroblasts in culture by some entirely unrelated mechanism. Nevertheless, use of *cis*-hydroxyproline is a simple technique which may be useful in preventing an overgrowth of fibroblasts, thus facilitating the preparation of primary cultures of other cell types. Other proline analogues which are incorporated into protein may be useful for the same purposes, although 0.2 mM (25 μg ml^{-1}) 3,4-dehydroproline was found not to inhibit the growth of L-929 fibroblasts[13].

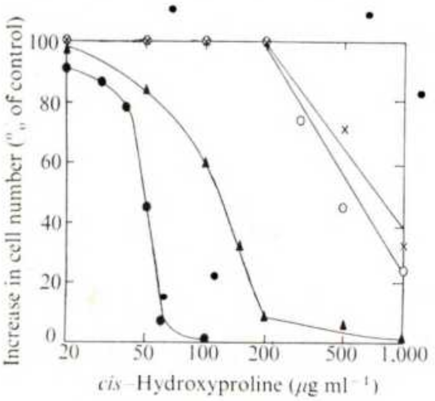

Fig. 2 Dose dependency of effect of *cis*-hydroxyproline on the growth of cells. Various amounts of *cis*-hydroxyproline were added to cultures at mid-log phase about 48 h after inoculation. In each case the experiment was terminated after the cell number in control cultures had doubled. Cells used were tendon fibroblasts (●); L-929 fibroblasts (▲); KB cells (○); and neuroblastoma cells (X).

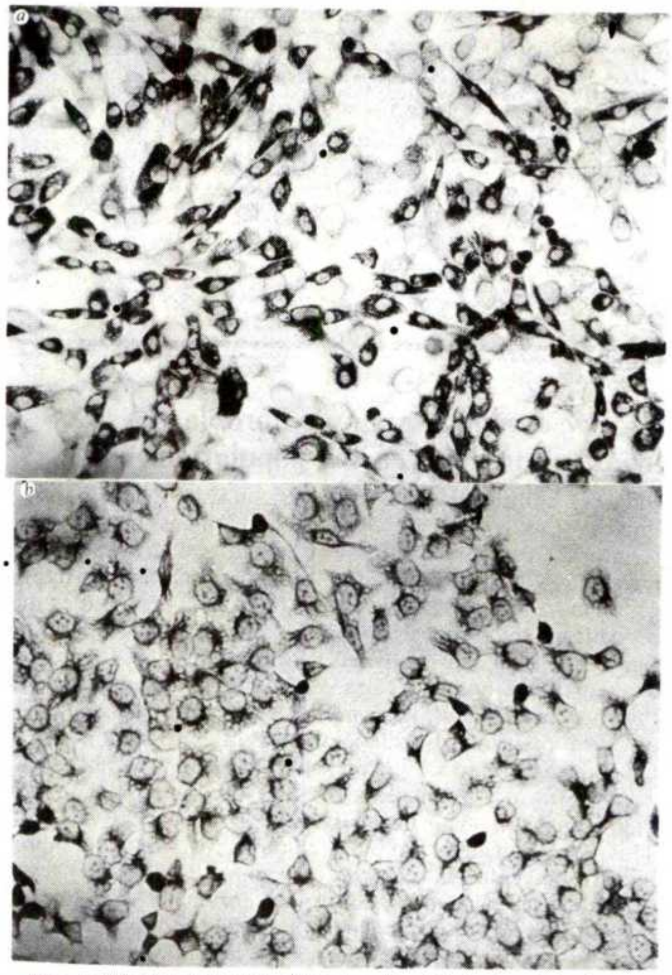

Fig. 3 Selective removal of tendon fibroblasts by *cis*-hydroxyproline from a mixed culture of tendon fibroblasts and KB cells. Equal numbers of tendon fibroblasts and KB cells were mixed and inoculated on coverslide inside a culture tube (Leighton type, Bellco) at a density of about 4×10^4 cm^{-2}. To half of the cultures 100 μg ml^{-1} of *cis*-hydroxyproline was added at the time of inoculation. The culture medium was changed every other day, and the cultures were maintained for 1 week. The cells on cover slides were fixed with ethanol–ether mixture, and then incubated with peroxidase conjugated to antibodies directed against chick prolyl hydroxylase. The samples were stained with 3,3′-diaminobenzidine as described before[9]. *a*, Photomicrograph of control sample. Tendon fibroblasts appear spindle shaped and are darkly stained. *b*, Photomicrograph of mixed cultures treated with *cis*-hydroxyproline. Only KB cells are seen; the few darkly-stained bodies probably represent dead cells.

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Prostaglandin E_2-9-ketoreductase as a mediator of salt intake-related prostaglandin-renin interaction

PREVIOUS studies have revealed a close relationship between the prostaglandin (PG) and the renin–angiotensin systems in the kidney[1–4] and we assume that both systems are intimately involved in the regulation of salt and volume homeostasis and of blood pressure. Changes in NaCl and water balance were found to be important determinants of the activity and function of the renin–angiotensin system[5] and it has been suggested that PG synthesis is also modified by these variables[2,6,7]. One factor influencing the pathway and, thus, the consequences of altered PG formation is the enzyme PGE_2-9-ketoreductase, which catalyses the interconversion of the antagonistically acting PGE_2 and $PGF_{2\alpha}$ (ref. 8). First direct evidence is presented here that PGE_2-9-ketoreductase activity is under the control of NaCl ingestion. We suggest that this enzyme is an important mediator of salt-intake-related prostaglandin–renin interaction.

PGE_2-9-ketoreductase activity has been demonstrated in the renal cortex and medulla of several species, including man and rabbit[9,10]. In our investigation in the rabbit, the effects of low and high NaCl ingestion on renal cortical and medullary PGE_2-9-ketoreductase and plasma renin activity were examined. Since urinary prostaglandin excretion is assumed to reflect renal prostaglandin biosynthesis[11], urinary PGE_2 and $PGF_{2\alpha}$ excretion were also measured (Table 1 and Fig. 1). At high NaCl intake, sodium excretion was nine times, but the urinary volume was only twice that at low NaCl intake. Plasma renin activity (PRA) was 12.9 ng ml^{-1} h^{-1} at low and decreased to 1.8 ng ml^{-1} h^{-1} at high NaCl intake. At low NaCl intake, urinary excretion of PGE_2 per day measured 1.9 μg and of $PGF_{2\alpha}$ 2.1 μg, giving a ratio of PGE_2 to $PGF_{2\alpha}$ of 1:1.1. PGE_2-9-ketoreductase activity in renal cortex was 0.61 ± 0.07 nM $PGF_{2\alpha}$ formed min^{-1} mg^{-1} (s.e.m., $n = 13$) and in the medulla 0.1 ± 0.01 (s.e.m., $n = 13$). At high NaCl intake, PGE_2-9-ketoreductase activity in the renal cortex increased threefold to 1.74 ± 0.13 (s.e.m., $n = 10$; $P < 0.001$) and in the medulla twofold to 0.19 ± 0.01 (s.e.m., $n = 10$; $P < 0.001$). At the same time urinary excretion of PGE_2 per day decreased to 0.53 μg, whereas that of $PGF_{2\alpha}$ remained practically unchanged at 2.43 μg, giving a ratio of PGE_2 to $PGF_{2\alpha}$ of 1:4.6.

The observed decrease in urinary PGE_2 excretion on high NaCl intake may result from either a decrease in renal PGE_2 synthesis, an alteration in PGE_2 metabolism at maintained synthesis, or both. The total amount of PGE_2 and $PGF_{2\alpha}$ excreted in urine decreased at high NaCl intake, suggesting a lower renal PG production. A reduction in formation or breakdown of PG endoperoxides, however, could only explain the lower PGE_2 excretion but not account for the unchanged $PGF_{2\alpha}$ excretion found on high NaCl intake. These findings can best be interpreted in terms of an altered pathway of PGE_2 metabolism on reduced PG synthesis. The increase of renal cortical and medullary PGE_2-9-ketoreductase activity, as demonstrated in this study during high NaCl intake, shifts intrarenal PG formation from PGE_2 to $PGF_{2\alpha}$, and could therefore explain the decrease in urinary PGE_2 excretion while $PGF_{2\alpha}$ excretion remains unchanged. Such a mechanism may be operative in man, since it has been observed in disease states in which variations in sodium and volume homeostasis are accompanied by changes in the PGE_2 to $PGF_{2\alpha}$ ratio[12].

The decrease of PRA after high NaCl intake confirms earlier observations[5] and reflects, in these conditions, a decrease in renin activity within the juxtaglomerular apparatus, which regulates synthesis and release of this enzyme. *In vitro*, renin release is increased by the PG precursor arachidonic acid, by PG endoperoxides or by PGE_2, whereas $PGF_{2\alpha}$ inhibits renin secretion[13,14]. $PGF_{2\alpha}$ may have this action also *in vivo*. Therefore, the decrease in PRA during salt-loading could result from the less opposed inhibitory action of $PGF_{2\alpha}$ on renin release. This assumption is supported by recent findings in man and dog, in which renin release decreased concomitant with increased sodium and $PGF_{2\alpha}$ excretion[15,16]. Thus, at high NaCl intake, the sodium-conserving renin–angiotensin system may be blocked by the relative increase in $PGF_{2\alpha}$ on reduced PG synthesis. In addition, distal nephron action of the antidiuretic hormone may be unopposed as a result of decreased PGE_2 formation[17,18]. These mechanisms would, at high NaCl intake, facilitate a high sodium load to be eliminated in a small urine volume, as demonstrated in our experiments (ninefold increase in urinary sodium excretion compared with twofold increase in urinary volume). At low NaCl intake the reverse was observed: PG synthesis may increase. PGE_2-9-ketoreductase activity is reduced, thereby increasing PGE_2 levels. Because of the stimulatory and relatively less opposed

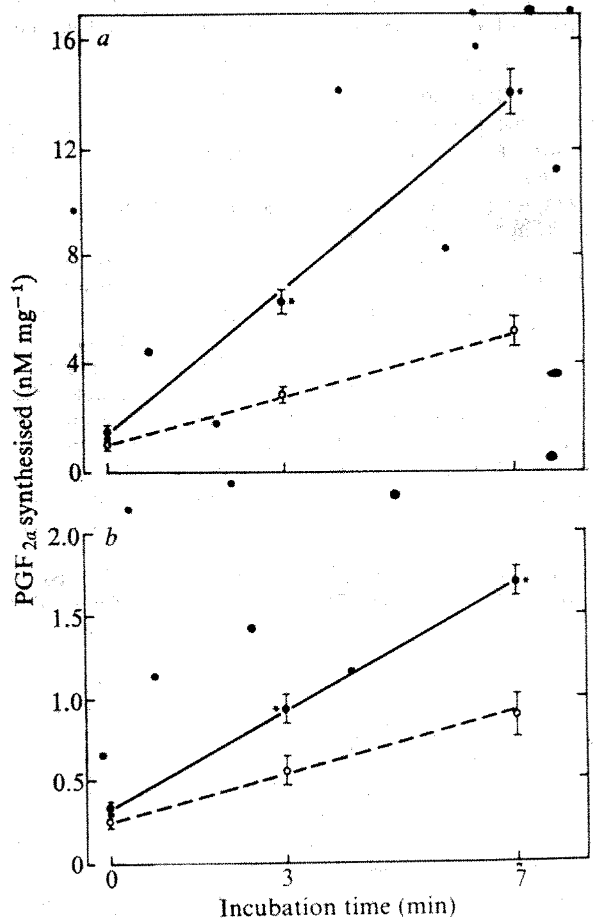

Fig. 1 Enzyme kinetic determinations of renal cortical (*a*) and medullary (*b*) PGE_2-9-ketoreductase activity of rabbits at low (○) and high (●) NaCl intake, respectively. Mean values are given as nM $PGF_{2\alpha}$ formed per mg protein (± s.e.m. $n = 13$ for low, $n = 10$ for high NaCl intake). NADPH-dependent PGE_2-9-ketoreductase activity was determined by the method of Stone and Hart[9] with minor modifications. Cortex and medulla of the removed kidneys were dissected at 4 °C. A 20% homogenate was prepared in 9.2 mM potassium phosphate buffer (*p*H 7.3) at a final concentration of 4 mM $MgCl_2$ and 0.1 mM 1,4-dithioerythritol (Merck, W. G.). The suspensions of cortex and medulla were centrifuged for 60 min at 100,000 *g*. The high speed supernatant fluids were dialysed for 24 h against 100 volumes phosphate buffer. Incubations were carried out at 37 °C using 200 μl supernatant as the source of PGE_2-9-ketoreductase, in a total volume of 600 μl phosphate buffer, at final concentrations of 1 mM $NADPH_2$ (Merck, W. G.) as cofactor and 1.2 mM PGE_2 (Upjohn, Kalamazoo) as substrate in excess (≥ fourfold K_M). At 0, 3 and 7 min, 100 μl of the mixture were removed. The reaction was stopped by adding 500 μl ice-cold Tris-HCl buffer (10 mM, *p*H 7.4, containing 0.1% gelatin and 140 mM NaCl), and heating the samples for 2 min to 100 °C. The amounts of $PGF_{2\alpha}$, enzymatically converted from PGE_2, were analysed by RIA (ref. 9). Reaction velocity of PGE_2-9-ketoreductase was calculated from the amounts of $PGF_{2\alpha}$ generated per minute and per milligram protein, determined by the method of Lowry *et al.* (ref. 25).

* $P < 0.001$. Level of significance between values obtained in rabbits on low and high NaCl diet was determined by the unpaired Student's *t* test.

Table 1 Effects of low and high NaCl intake on renal parameters in rabbits

NaCl intake (per 100 g food)	$U\dot{V}_{Na+}$ (mmol d^{-1})	$U\dot{V}$ (ml d^{-1})	Urinary PGE_2 excretion (μg d^{-1})	Urinary $PGF_{2\alpha}$ excretion (μg d^{-1})	PRA (ng ml^{-1} h^{-1})
Low: (13) 0.25 g	6.1 ± 0.4	146 ± 8	1.9 ± 0.1	2.1 ± 0.2	12.9 ± 1.2
High: (10) 2.50 g	53.8 ± 4*	295 ± 11*	0.53 ± 17*	2.43 ± 0.3	1.8 ± 0.3*

The effects of a sodium-depleted (0.25 g NaCl per 100 g food; or sodium-enriched (2.5 g NaCl per 100 g food) diet (Altromin, W. G.) on daily urinary sodium excretion ($U\dot{V}_{Na}$) and urinary volume ($U\dot{V}$), PGE_2 and $PGF_{2\alpha}$ excretion and PRA in cross-bred rabbits. Values are given as mean ± s.e.m. Numbers in parentheses indicate the number of animals. Experiments were carried out in rabbits kept in metabolic conditions for 10 d on either diet with drinking water *ad libitum*. Urinary parameters and PRA were measured in conscious animals the day before the kidneys were removed under pentobarbital anaesthesia for determination of renal PGE_2-9-ketoreductase activity. PRA was determined by radioimmunoassay (RIA) of angiotensin I as described previously[2,3]. PGE_2 and $PGF_{2\alpha}$ were analysed after previous extraction and group separation of the urinary PGs on silicic acid columns (Unisil, Clarkson Chemicals, Pennsylvania)[11,12]. PGE_2 was measured in parallel by bioassay of PGE_2 (ref. 24) and by RIA using a PGB-specific antibody after conversion of the PGE fraction to PGB[12]. The correlation between values obtained with both methods was highly significant ($y = 0.8x + 4.9$; $r = 0.94$). $PGF_{2\alpha}$ excretion was determined using a commercially available $PGF_{2\alpha}$ antibody (Calbiochem, Pennsylvania)[11,12].

* $P < 0.001$. Level of significance between values obtained in rabbits on a low and high NaCl diet was determined by the unpaired Student's *t* test.

action of PG endoperoxides or PGE_2 on renin release, the stimulated renin activity could then initiate the sodium-conserving mechanism[5]. In addition, at low NaCl intake, increasing PGE_2 levels may enhance distal tubular sodium reabsorption[18], and further support the effectiveness of the stimulated sodium-conserving renin-angiotensin system.

We therefore propose the concept that the activity of renal PGE_2-9-ketoreductase is dependent on NaCl ingestion or some factor related to salt intake. Our results suggest that this enzyme is intimately involved in adjusting the activity of the renin-angiotensin system and distal nephron function in response to varying levels of NaCl intake. Thus, renal PGE_2-9-ketoreductase may play a pivotal role in the regulation of NaCl and water balance. Finally, the demonstration of PG synthesis and of PGE_2-9-ketoreductase activity in blood vessel walls[19,20] may provoke new speculations on the finding that vascular reactivity and blood pressure are influenced by the state of NaCl balance (refs 21 and 22).

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Myopia and eye enlargement after neonatal lid fusion in monkeys

THE aetiology of myopia has been studied mainly by investigating the distribution of refractive errors in human populations[1]. No clear conclusion has emerged, however, so the prevailing clinical attitude is that myopia can neither be prevented nor cured, but only corrected with appropriate lenses. The mechanism of myopia can be rationally analysed only if a suitable animal model is found for this refractive condition. In the literature, there are only two examples of experimental myopia. Levinsohn[2] claimed that a high degree of myopia develops in young monkeys when the body is kept elevated above the head for long periods of time; his results, however, have been questioned[3,4]. Young[5] induced myopia by exposing monkeys to a restricted visual space, but the refractive error thus obtained was relatively small. During investigations on monocular visual deprivation in monkeys[6] it was noted that the eye in which the lids had been closed by suture at birth developed a high degree of myopia. We report here a study of the effects of neonatal lid fusion on the refractive state and anatomy of the macaque monkey eye.

In 10 macaque monkeys (*Macaca mulatta*, *Macaca arctoides*) monocular or binocular lid fusion was performed at various times after birth. The surgical technique was as follows: under general anaesthesia, the margin of both lids was excised lateral to the lacrimal papilla. The conjunctiva was removed for an additional couple of millimetres to prevent its ingrowth into the wound and the cut surfaces of the lids were approximated by silk mattress sutures from the lateral canthus to the lacrimal papilla. Sutures were removed 1–2 weeks after surgery, when the lids had fused and appeared as a thin, translucent diaphragm that attenuated light by about half a log unit. After a variable period of lid fusion (19 d to 26 months) the palpebral fissure was re-established. Accommodation was paralysed with 2 drops of a 1% solution of homatropine and the refraction of both eyes determined by using a streak retinoscope and hand-held trial case lenses. The corneal curvature was measured with a keratometer and the fundus was examined. At various times after lid opening, animals were refracted again, used for electrophysiological studies on the visual pathways and finally perfused through the heart with 10%

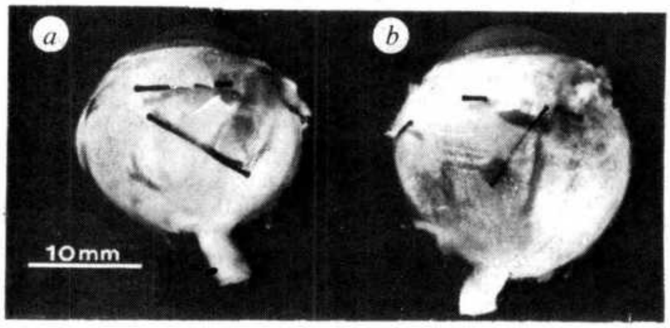

Fig. 1 Eyes of a rhesus monkey in which the lids on the right (*b*) were fused at the age of 2 weeks and opened 18 months later (experiment 5 in Table 1). Suture threads mark the insertions of the extrinsic ocular muscles. The left eye (*a*) was normal.

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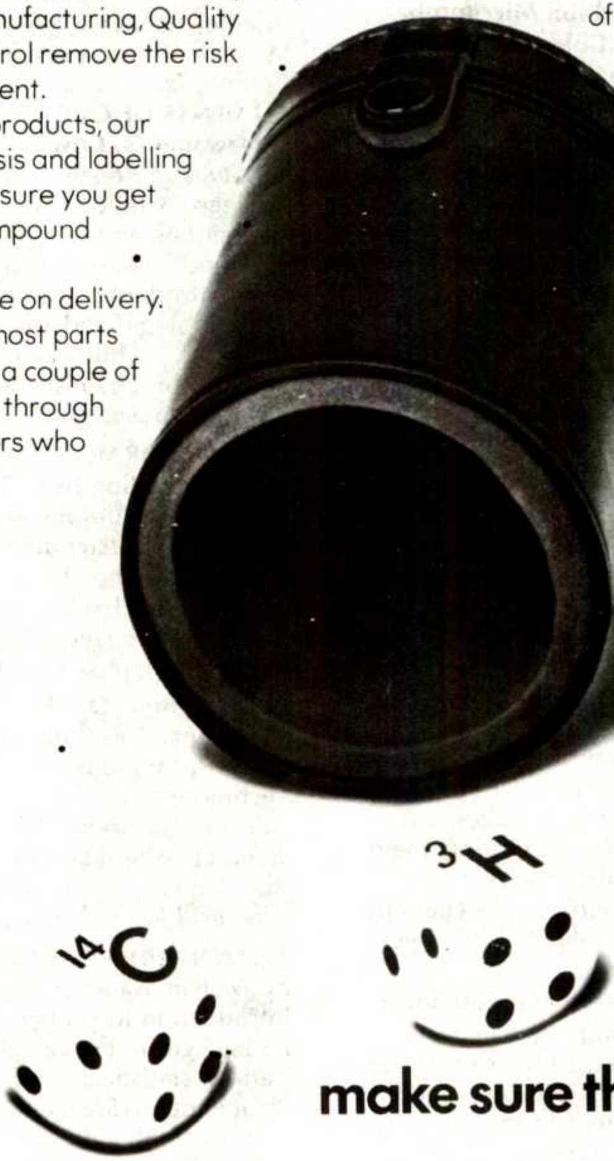

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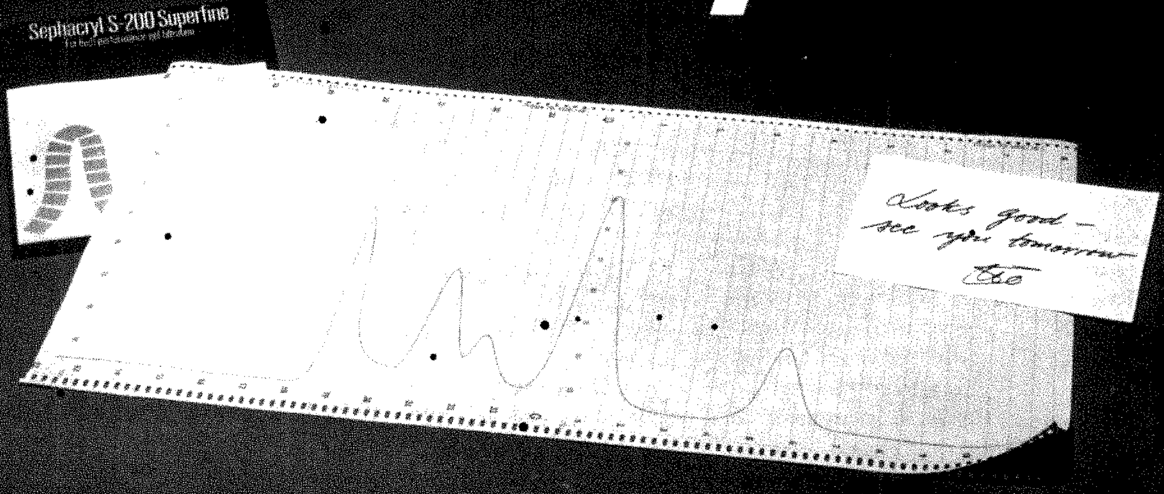

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Table 1 Interocular difference in refraction and axial length following neonatal lid fusion in macaque monkeys

Experiment no.	Age at lid fusion	Duration of lid fusion	Difference in refraction (dioptre)	Difference in axial length*	*Macaca* species
1	11 d	19 d	−1.0	—	*mulatta*
2	Birth	6 weeks	−2.75	1.089	*mulatta*
3	11 d	6 months	−7.0	1.077	*mulatta*
4	4 d	12 months	−9.5	1.101	*arctoides*
5	2 weeks	18 months	−13.5	1.208	*mulatta*
6	6 weeks	15 months	−8.5	1.108	*mulatta*
7	12 months	26 months	−4.5	1.010	*arctoides*
8	mature	17 months	0	—	*mulatta*

*Expressed as a ratio between axial length of the myopic eye and axial length of the normal eye.

formalin. The eyes were enucleated, measured and processed for light microscopy; in some animals, the extraocular muscles were prepared for microscopy.

To illustrate the effects of monocular lid fusion on the refraction and the anatomy of the eye, we describe an experiment in which results were striking and a comprehensive morphological analysis was performed (experiment 5 in Table 1). Lids were sutured in the right eye of a rhesus monkey at the age of 2 weeks and the fusion maintained for 18 months. Upon re-establishing the palpebral fissure, a normal conjunctival sac was found and the appearance of the exposed surface of the eye globe was identical to the control side. The interocular difference in curvature of the anterior corneal surface was slight (average refractive power in the right eye 51 dioptres, in the left eye 52.5 dioptres). Retinoscopy showed that the dioptric media were normally transparent and that the right eye was 13.5 dioptres more myopic than the left eye. The appearance of the fundus was normal; furthermore, the physiological experiments indicated that the lateral geniculate bodies received normal retinal input from both eyes.

At autopsy, the right eye was considerably larger than the left eye (Fig. 1). Various eye dimensions were measured and some of the results are diagrammatically represented in Fig. 2. The most remarkable finding was a 21% increase in the axial length of the right eye, as measured from the summit of the cornea to the projection of the fovea on the sclera (corresponding to the point of entry of the lateral long posterior ciliary artery and long ciliary nerve into the eye globe); the increase in equatorial diameter of the right eye was 7% only. Corneal diameters and distance of the insertions of the rectus muscles from the limbus were identical in both eyes, whereas the linear distance of the muscle insertions from the optic nerve was 23% larger in the right eye. Finally, the distance of the fovea from the centre of the optic disk was 18% larger in the myopic eye.

Histological examination showed that the posterior sclera was thinner in the right eye. Thickness of the cornea, choroid and retina were identical in both eyes. Also identical were the cross-sectional area of the ciliary muscle, the diameter of the optic nerve, the structure of the optic nerve head, and the cross-sectional area of the extrinsic ocular muscles.

Thus, neonatal lid fusion caused an elongation of the eye globe, characterised by distension of the postequatorial hemisphere and attenuation of the posterior sclera. All remaining ocular structures seemed unchanged.

Table 1 lists the results of all of our monocular lid fusion experiments. It shows that the degree of myopia caused by lid fusion increased with the duration of closure, that the error of refraction was less pronounced when lids were fused in older animals, and that there was no change in the adult. It should be noted that there was some variability in the response of different individuals to lid fusion, but that all animals in which lids were fused early in life became myopic.

In order to establish whether the refractive error was caused by a functional imbalance between the eyes, lids were fused bilaterally in a rhesus monkey at the age of 5 weeks. Eleven months later, −11-dioptre myopia was present bilaterally and both eyes were equally enlarged. In another instance, lids were fused in the right eye of a rhesus monkey at the age of 1 week and opened 12 months later; a −10-dioptre myopia was present in the sutured eye. Lids were subsequently fused in the left eye and opened 13 months later; this eye only developed a −6-dioptre myopia. The axial length was 23.25 mm in the right eye and 22 mm in the left eye (the average axial length of the eye in the mature rhesus monkey was 19.34 mm ±0.14 s.e.m., measured in 15 animals, right and left eyes pooled). These experiments confirm that larger refractive errors are obtained in younger animals and demonstrate that the development of myopia in each eye is independent of the functional state of the other eye. Finally, it is important to mention that myopia persists unmodified for years after lid opening.

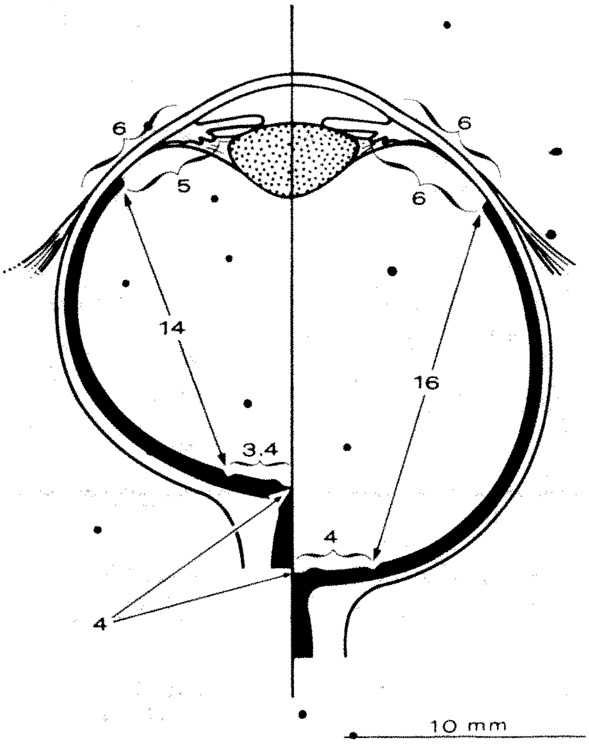

Fig. 2 Diagram illustrating the effects of neonatal lid fusion on various eye dimensions. The temporal halves of the eyes are juxtaposed. (Experiment 5 in Table 1.)

The myopia caused by lid fusion is clearly of the axial type, for the corneal refraction is not affected significantly.

The fact that the refractive error is larger when lid fusion is performed early in life, suggests that this type of myopia represents a derangement of the development of the eye. Presumably, neonatal lid fusion somehow accelerates and accentuates a mechanism which operates normally in postnatal life. Supporting this interpretation is the finding that the postequatorial segment of the globe elongates, whereas the anterior segment is relatively unaffected: in fact, it is known that in humans the cornea changes but little after birth, whereas the axial length increases dramatically[1]. The simplicity and effectiveness of the technique and the fact that one eye can be used as a control, indicate to us that monocular myopia caused by neonatal lid fusion in monkeys represents a useful animal model for myopia in general, and that a study of its pathogenesis may provide insight into the development of this refractive error in humans. After a presentation of our results[7], G. K. van Noorden reported inconsistent changes in refraction following lid fusion in neonatal rhesus monkeys; we have no explanation for the discrepancy.

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Remyelination of CNS axons by Schwann cells transplanted from the sciatic nerve

SCHWANN cells can remyelinate axons normally myelinated by oligodendrocytes, after either primary demyelination[1–3] or suppression of myelination by X irradiation at the time of myelination[4,5]. It has been suggested that the glial limiting membrane normally inhibits Schwann cell invasion of the CNS (ref. 2) and its reformation inhibits uncontrolled invasion by Schwann cells once this phenomenon has been initiated[1,2]. The Schwann cells in these situations are thought to arise from the nerve roots. I undertook experiments to determine whether it is possible to suppress remyelination following primary demyelination in an experimental animal. If so the resulting demyelinated axons would be similar to those present in the multiple sclerosis plaque, that is they would remain demyelinated unrepaired by glial or Schwann cells. Using such a lesion it would then be possible to establish if Schwann cells obtained from a source outside the central neuraxis would remyelinate these axons. In the past, attempts at transplanting peripheral nervous tissue into the central nervous system have shown very limited invasion of peripheral elements into the neuropil of the CNS (refs. 6, 7) and it was hoped that the presence of large numbers of demyelinated axons would act as a stimulus for Schwann cell invasion[8]. In addition, intraspinal injections of lysolecithin facilitate Schwann cell invasion of the CNS from local sources[2–9]. By abolishing this intrinsic Schwann cell remyelination potential and by substituting an extrinsic source of Schwann cells it might be possible to determine if persistently demyelinated axons in the CNS, similar to those in multiple sclerosis plaques, can be repaired by outside interference. I report here the preparation of areas of demyelination in rats and cats which remyelinated only in the presence of added Schwann cells.

The first step was to induce an area of primary demyelination which was incapable of being remyelinated with the cells available within the central neuraxis, that is, by oligodendrocytes or by Schwann cells derived from the spinal roots.

Intraspinal injection of lysolecithin (lysophosphatidyl choline) induces an area of primary demyelination at the site of injection in rats and cats[2–9] and all the demyelinated axons are subsequently remyelinated either by oligodendrocytes or Schwann cells depending on their location within the lesion. In order to suppress this remyelination the spinal cord caudal to Th_{10} spinal level was irradiated 2 weeks before the injection of lysolecithin with 4,000 rad of X rays (from a Marconi radiotherapy machine using 250 kV, 15 mA, 0.5 mm Cu, 1 mm Al, f.s.d. 50 cm for cats and 32 cm for rats, under pentobarbitone anaesthesia). In rats and cats subjected to this procedure there was no evidence of remyelination when they were subsequently killed (Table 1 and Fig. 1). These experiments therefore established that X irradiation with 4,000 rad before demyelination suppresses both oligodendrocyte and Schwann cell remyelination, leaving large numbers of persistently demyelinated axons in the spinal cord (Fig. 1).

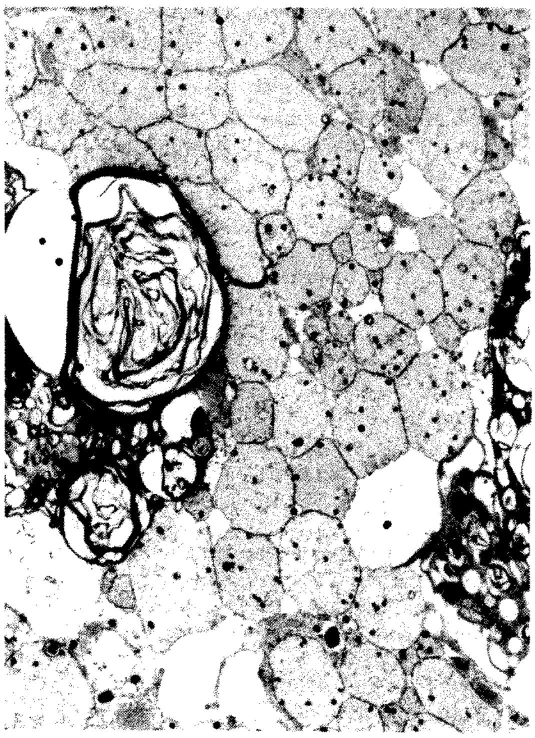

Fig. 1 Persistently demyelinated axons in the dorsal column following lysolecithin injection into the X-irradiated spinal cord (rat R53). (×4,400.)

In the second series of experiments with these two species a 1.0-cm length of dissociated sciatic nerve was placed over the injected area to provide a source of viable Schwann cells. The experimental procedure was as follows. At the time of spinal cord irradiation the cat or rat sciatic nerve was crushed at the mid-thigh level. A laminectomy was carried out 2 weeks later in the region of Th_{12-13}, the dura were incised and lysolecithin was injected into the dorsal columns as before[2–9]. In most cases 3–5 μl of a 1.0% solution of lysolecithin (Sigma) in saline were injected in up to three sites using a hand held glass micropipette attached to an Aglar syringe (Wellcome reagents) by means of fine polythene tubing. A 1.0-cm length of sciatic nerve was then excised distal to the previously made crush, desheathed and slightly teased in tissue culture media

Table 1 Relationship between experimental treatment and remyelination

	Experimental treatment				Type of remyelination			
Animal	Lysolecithin	Iradiation	Transplantation	Survival time	Oligodendrocyte remyelination	Schwann cell remyelination	Persistent demyelination	Comments
Rats								
R 68	T	O	O	7 W	+ + +	+	–	Small deep lesion
R 69	T	O	O	7 W	+ +	+ + +	–	Several large lesions
R 52	O	T	O	7 W	–	–	–	No abnormalities
R 53	T	T	O	18 d	–	–	+ + +	
R 54	T	T	O	27 d	–	–	+ + +	
R 55	T	T	O	19 d	–	–	+ + +	
R109	T	T	T	33 d	–	+ + +	+	Ventral lesion persistent demyelination
R110	T	T	T	24 d	–	+ + +	+	Ventral lesion persistent demyelination
R111	T	T	T	24 d	–	+ + +	+	
R113	T	T	T	33 d	–	+	+	Very small lesion
Cats								
R108	T	T	O	20 d	–	–	+ + +	
R128	T	T	O	7 W	–	–	+ + +	
R132	T	T	O	8 W	–	–	+ + +	
R129	T	T	T	7 W	–	+ +	+ +	
R130	T	T	T	8 W	–	+ + +	+	
R131	T	T	T	6 W	–	–	+	Much Wallerian degeneration and fibrosis
R133	T	T	T	8 W	–	+ + +	+ +	

T, Treatment given; O, treatment omitted; –, none; +, some; + +, many; + + +, very many; W, week; d, days.

under a dissecting microscope. The teased mass was placed over the exposed surface of the spinal cord and tucked under the dura. In some cases it was also pushed into the substance of the cord in the region of the lysolecithin injections. The wound was closed and animals were killed at various times after these procedures (Table 1) by aortic perfusion with 4% glutaraldehyde in phosphate buffer under pentobarbitone anaesthesia. The cord was removed and 1–2-mm transverse slices from the injected area were post fixed in osmium tetroxide and embedded in Araldite. The effects of transplantation were assessed using 1-μm sections stained with alkaline toluidine blue.

In all animals which received nerve transplants, with the exception of cat R131, Schwann cell remyelination was present in the areas demyelinated by the injection of lysolecithin (Table 1) (Fig. 2). The extent of Schwann cell remyelination varied but in general was greater in the rats than the cats. Not all demyelinated axons were remyelinated, which in the rats may be related to the short survival times; however in the cats this failure to remyelinate completely may be due to other factors because the persistently demyelinated axons occurred deeper in the cord than those remyelinated by the Schwann cells. In the rats lesions in the ventral columns present in animals R109 and R110, showed no evidence of remyelination confirming the results found in the non-transplanted animals that the dose of irradiation used suppresses inherent remyelination.

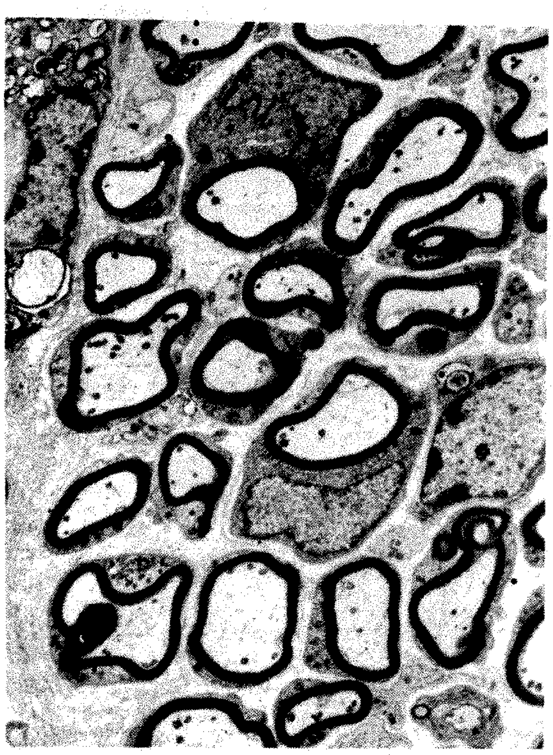

Fig. 2 Schwann cell remyelination following transplantation of sciatic nerve (rat R111). ×4,400.

These experiments show that it is possible to prepare small localised areas of primary demyelination in experimental animals which do not remyelinate (Fig. 1); in this they resemble the persistently demyelinated plaques of multiple sclerosis. When a source of Schwann cells is provided the axons are remyelinated by these cells (Fig. 2); this is most marked with axons close to the transplanted cells but more distant axons may remain demyelinated. Although the experimental system used to demonstrate this phenomenon is grossly abnormal, indeed is potentially lethal to the animal, it does show that it is possible to repair areas of demyelination which if left alone would remain demyelinated. The implication of this observation to human neurology must be considered, and factors which influence Schwann cell behaviour once they gain entry into the CNS should be studied. It remains to be determined if the presence of Schwann cells within the CNS is ultimately beneficial or harmful.

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Anaesthetic and convulsant ethers act on different sites at the crab neuromuscular junction *in vitro*

ATHOUGH volatile general anaesthetics, of the so called structurally non-specific type, have been shown to affect synaptic transmission[1,2], the underlying mechanisms are unknown. In view of their oil solubility, it has been suggested that these anaesthetics owe their depressant effect to the ability to induce non-specific disordering of membrane lipids[3,4]. But some equally oil-soluble and structurally non-specific ethers are potent convulsants rather than anaesthetics[5] and theoretical considerations suggest[6] that the two groups act at different sites in the nervous system and therefore are more specific in action than assumed so far. In support of this, we now report that each of a pair of representative agents, methoxyflurane, an anaesthetic and hexafluorodiethylether (HFE, Indoklon, Ohio Medical and Surgical Suppliers), a convulsant, have different effects on excitatory or inhibitory transmission at the crab neuromuscular junction. We have found that in the adductor muscle of the crab, *Ocypoda cursor*, which contains both glutamate and γ-amino-butyrate (GABA) receptors, methoxyflurane blocks the action of glutamate but not of GABA, while HFE blocks the effect of GABA but not of glutamate.

Muscle fibre cells were impaled with two micropipettes filled with 2 m KCl and having a resistance of 1–3 MΩ. One electrode recorded voltage and the other injected current. Usually, hyperpolarising current pulses of 100–500 nA and 100–200 ms were used to measure the effective input resistance of the cells. The voltage, V, and current, I, traces were amplified suitably and recorded on chart paper (Grass Polygraph model 79c). Membrane conductance, G, was computed as $G = I/V$. The preparation was perfused continuously with medium containing the desired drug at 22 ± 2 C. The working concentrations of methoxyflurane and HFE were set at their clinically effective blood levels[11,12].

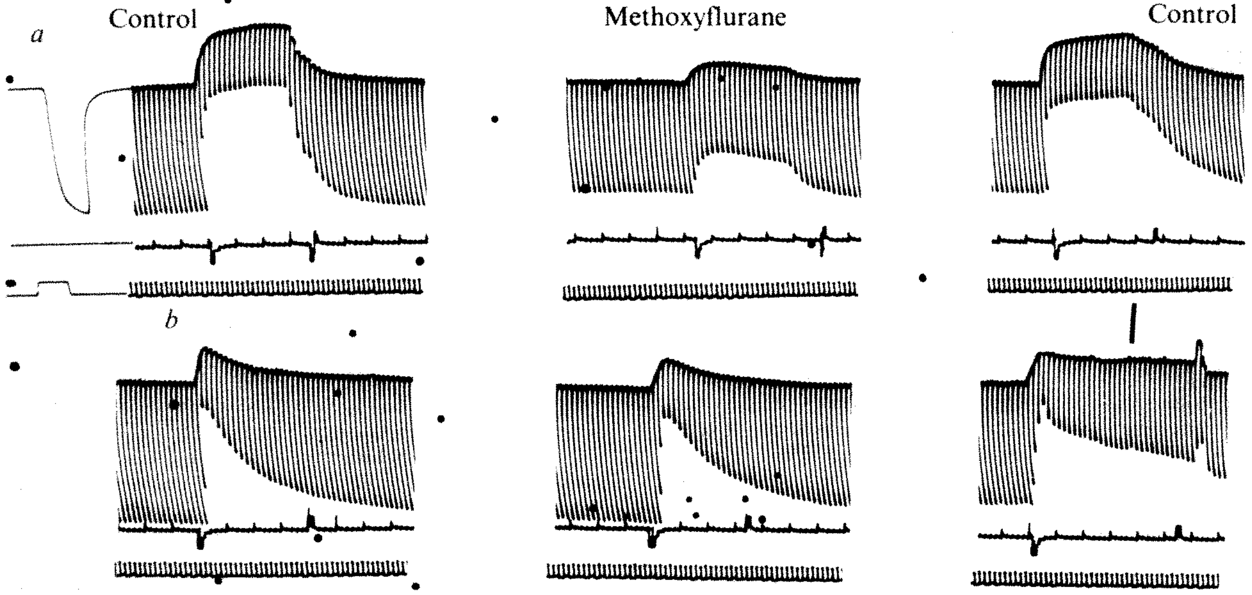

Fig. 1 Intracellular recording of the effect of 10^{-4}M methoxyflurane, $CHCl_2CF_2OCH_3$ on glutamate-induced *(a)* and GABA-induced *(b)* conductance change in a cell from the adductor muscle of *Ocypoda cursor*, resting potential −75 mV, input impedence 60 kΩ. *a*, Voltage changes caused by square current pulses of 200-nA amplitude and 100-m duration in the following order: with 5×10^{-5}M glutamate; with glutamate and methoxyflurane; and with glutamate after removal of methoxyflurane. *b*, In the same sequence, voltage changes in the presence of 5×10^{-5}M GABA; GABA and methoxyflurane and GABA after removal of methoxyflurane. Time scale: 1 and 5-s marks. Downward deflection on time scale indicates start of transmitter application; upward deflection indicates its termination. Calibration, 5 mV and 500 nA. In these and subsequent experiments, the composition of the bathing solution was, in mM: NaCl, 453; KCl, 12.8; $CaCl_2$, 34; $MgCl_2$, 4.9; Tris buffer, 2. Muscle fibres were dissected and mounted according to Parnas *et al.*[13].

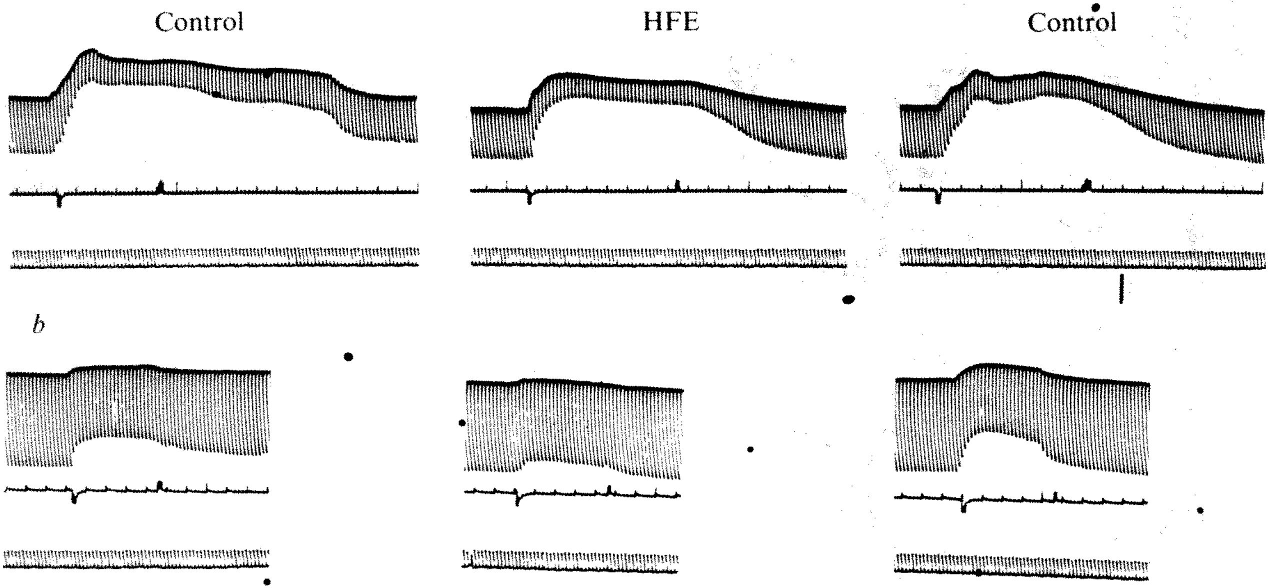

Fig. 2 Intracellular recording showing the effect of 10^{-4}M HFE, $CF_3CH_2OCH_2CF_3$, on glutamate-induced *(a)* and GABA-induced *(b)* conductance change in a cell from the adductor muscle of *Ocypoda cursor*, resting potential −78 mV and input impedence 25 kΩ *(a)* and 45 kΩ *(b)*. *(a)* and *(b)*, Voltage changes are shown in the same order and the deflections have the same significance as in Fig. 1. Calibration, 5 mV and 300 nA. Experimental conditions were the same for Fig. 1.

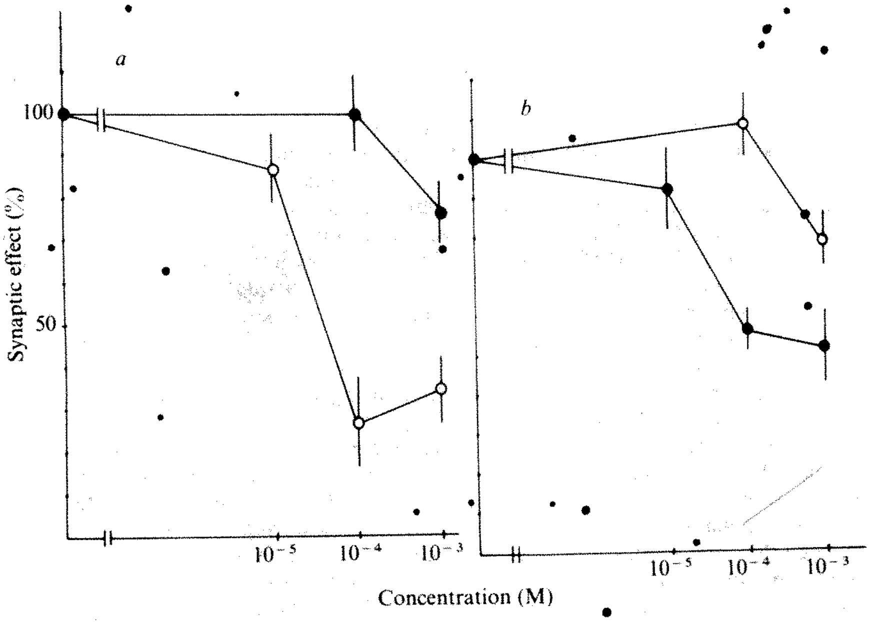

Fig. 3 Dose dependance of the depressing effect of methoxyflurane (○) and HFE (●) on glutamate-induced *(a)* and GBA-induced *(b)* conductance change. The ordinate is percentage change with respect to control. Bars indicate standard deviation. Data for 10^{-4} M are averages from eight cells; those for 10^{-3} M and 10^{-5} M are averages from the five cells each.

Figure 1 shows results from a typical experiment with 10^{-4} M methoxyflurane. When at first the preparation was perfused with 5×10^{-5} M glutamate only, the muscle was depolarised and its input impedence decreased markedly. But after exposure of the cell to methoxyflurane for 5 min, followed by methoxyflurane and glutamate, the latter elicited a much reduced response. Eventually, when methoxyflurane was washed out, the original response to glutamate could be fully restored. This blocking effect of methoxyflurane was seen in eight cells where glutamate-induced conduction was reduced to $26\pm4\%$ of control. In identical conditions, methoxyflurane did not block the action of GABA, which induced slight depolarisation and a marked reduction in membrane resistance. In eight experiments, the effect of GABA in the presence of methoxyflurane remained at $108\pm3\%$ of control.

Opposite results were obtained with the convulsant, HFE (Fig. 2). No effect of 10^{-4} M HFE was recorded on the action of glutamate. Thus, glutamate-induced conduction in the presence of HFE was $99\pm3\%$ ($n=8$) of control. On the other hand, 10^{-4} M HFE blocked the synaptic effect of GABA, and GABA-induced conduction was reduced to $56\pm2\%$ of control ($n=8$). This apparent specificity of action in one type of synaptic transmission rather than in the other became less pronounced with higher concentrations of drugs. Thus, at 10^{-3} M, either drug suppressed the action of both glutamate and GABA (Fig. 3); methoxyflurane remained more active with respect to glutamate while HFE was more active with respect to GABA.

We infer that the blocking mechanisms of these drugs at the molecular level are basically the same, but that they differ in potency with respect to function. Measurements in the exophase show that the equipotent molar ratio of methoxyflurane to HFE is about 1:100 in depressing glutamate transmission but 100:1 in depressing GABA transmission. Glutamate and GABA are believed to be excitatory and inhibitory transmitters in the crab, respectively[7]; and probably they have similar functions in the mammalian central nervous system (CNS)[8,9]. Extrapolated to CNS receptors[2-10], our results may explain why methoxyflurane is an anaesthetic whereas HFE is a convulsant. Also, the specificity of action of each agent seems to be incompatible with a model whereby the drugs produce a non-specific disordering of bulk membrane lipids[3,4]. Rather, they seem to affect distinctive sites, probably functionally different proteins embedded in the same membrane.

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Pathways for hydraulically and osmotically-induced water flows across epithelia

EVIDENCE linking an extracellular shunt pathway[1] for ions with intercellular junctions[2], with reports that those junctions are permeable to large molecules such as sucrose[3], inulin[3], lanthanum hydroxide[4] and horseradish peroxidase[5] in various fluid-transporting epithelia suggests that water could also traverse this pathway. There is evidence, moreover, for a high hydraulic-conductance pathway across gastric mucosa[6] and rumen[7] if hydrostatic pressure is the driving force. Yet, except for the kidney proximal tubule[8], measurements of osmotically-induced water flows across several 'leaky' epithelia have not in general yielded values[9] consistent with a high-conductance pathway. It has been argued, also, that the extracellular shunt is not a significant route for osmotic flow in the gallbladder[10]. To investigate these apparent discrepancies, we have measured the hydraulic (Lp) and osmotic (P_{os}) conductances of rabbit corneal endothelium. We report here that a large conductance for both hydraulic and osmotic flows either is present or can be demonstrated in appropriate conditions, and that its value is of the order expected for bulk water flow across the intercellular junctions.

The techniques for the dissection and mounting of the preparation, and the bathing medium's composition have been described before[11]. The corneal epithelium was scraped off. The rates of transendothelial fluid movement and the corresponding steady-state values of *Lp* and P_{os} were determined by an automatic technique[12] which maintains the volume of the outside compartment constant. The transient rates from which P_{os} at zero time was extrapolated were determined by microscopic measurements of corneal thickness[13].

The effect of a hydrostatic pressure difference (ΔP) on the rate of fluid movement was determined as described in Fig. 1. The basal rate of fluid transport was usually measured with a ΔP of 5 or 10 cm H_2O. After each change in ΔP there was a transient, followed by a period of reasonable stability. The effects were reversible during the 5–6 h of useful life of the preparation, then *Lp* increased progressively (Fig. 1), presumably due to cell deterioration. The long transients (Fig. 1) were attributed to changes in the thickness of the supporting stroma with ΔP. Evidence came from separate observations of isolated stroma; endothelium was scraped off and silicone oil (20 cs) filled the inside (or aqueous) chamber, which limited water movements to the stromal-outer chamber interface. Transient changes in rate of water movement due to changes in ΔP had the same amplitude and time course as those seen with endothelium present.

The results of all technically acceptable experiments are plotted in Fig. 2, and show that the flow is linear with the ΔP for most of the range examined. At the low end of that range there is a trend towards an increased dependence of flow on ΔP, but this point could not be substantiated by reversing the ΔP because of technical limitations. For the linear interval, *Lp* was estimated from Fig. 2 to be 83±11 μm s^{-1} (V_w at 37 °C: 1.81 cm^3 m^{-1}). 'Edge damage' was excluded as an influence in this value by mounting the preparation in the chamber, then clamping it with a cylindrical plug which left exposed only a 0.1-mm peripheral annulus. No leak was detected across this preparation even with a ΔP of 150 cm H_2O. To account for possible solute polarisation in the unstirred layers on both sides of the tissue, which could have led to an underestimation of *Lp* (ref. 14), we assumed that an increase in ΔP induced an outward flow which diminished the apparent inward flow due to transport. Unstirred layer widths and NaCl diffusion coefficients were either taken from the literature or assumed (inside: $\delta_1 = 350$ μm; $D_1 = 1.54 \times 10^{-5}$ cm^2 s^{-1}; outside: $\delta_2 = 850$ μm; $D_2 = 1.0 \times 10^{-5}$ cm^2 s^{-1}); taking for the basal rate of fluid transport a value of $Jv = 4.5$ μl h^{-1} cm^{-2} at a ΔP of 10 cm H_2O (Fig. 2), substituting into $C = C_{bulk} \exp(-Jv\,\delta/2D)$ (see later) for each interface and using $\sigma_{NaCl} = 0.6$ (ref. 15) the corrected *Lp* is 114 μm s^{-1}.

To determine P_{os}, inward flow was induced by making the inside solution hypertonic with sucrose, while outward flow was induced by making both sides initially 80 mOsm hypertonic with sucrose and then lowering the tonicity inside. The results summarised in Fig. 3 show that the steady-state osmotic flows are not linear with the osmotic pressure difference ($\Delta\pi$), just as is the case in other epithelia[9]. These steady-state osmotic flows were, however, preceded by transients, which prompted an investigation of the osmotic flows obtained shortly after the imposition of a $\Delta\pi$. The results in Fig. 4 show that for the range $10 < \Delta\pi < 80$ mOsm the initial rate of osmotic flow is linear with $\Delta\pi$. The slope for the linear region (Fig. 4) is 166±12

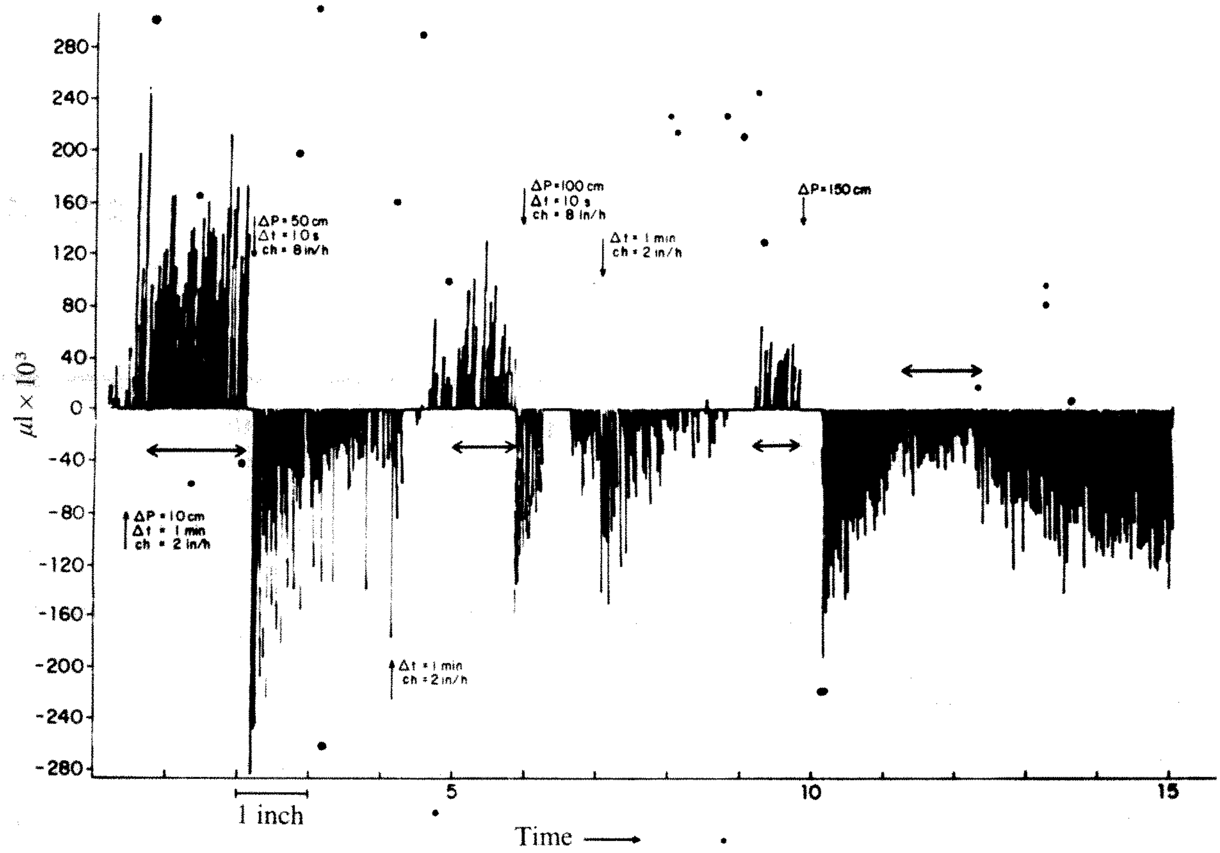

Fig. 1 Photograph of an experimental record. The height of the bars corresponds to the volume of fluid that traverses the preparation and is subsequently reinjected into (or aspirated from) the outside compartment by a stepping-motor-driven syringe. The recorder is reset to zero at time intervals Δt, so that a graphical display of the rate is obtained. The preparation was supported by a hemispherical stainless steel net, against which it was held by hydrostatic pressure applied on the inside (aqueous side). Chart markings are at 1-inch intervals; changes in ΔP, Δt and chart speed are shown at arrows. Positive ordinate denotes inward flow (from stroma to aqueous) and vice versa. In this experiment most of the stroma was dissected away. Average rates were computed from an accumulator for the periods denoted by horizontal bars. $T = 36.80 \pm 0.01$ °C; area of preparation = 175 mm^2.

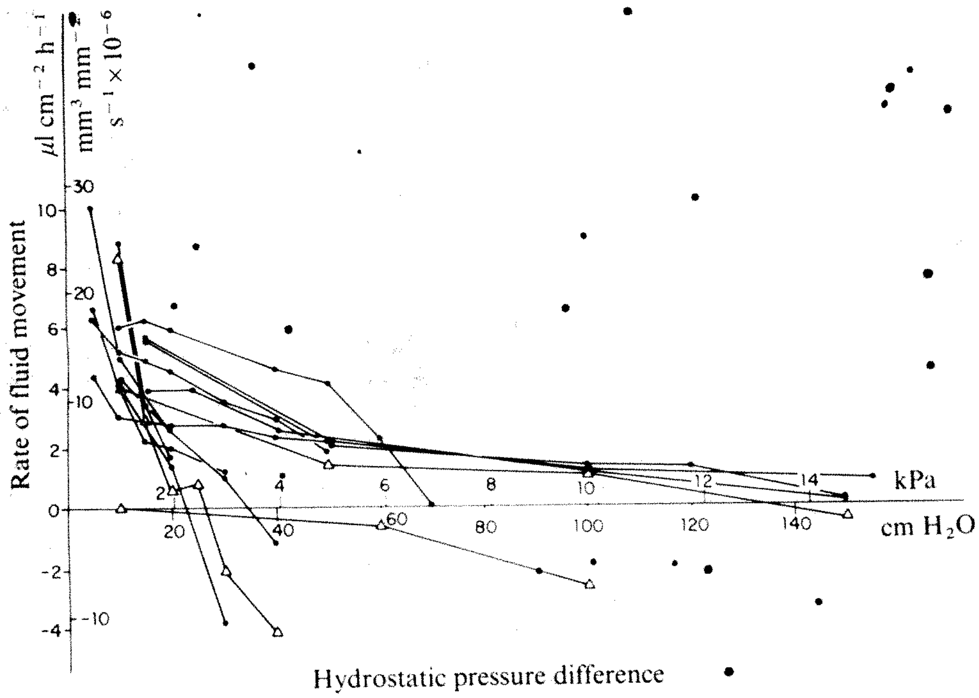

Fig. 2 Rate of transendothelial fluid movement with different values of hydrostatic pressure difference across that layer. Each curve represents an experiment; ●, the usual type: △, experiments in which most of the stroma was dissected away. Direction of flow and applied pressure as in Fig. 1. (kPa: kilopascals.)

µm s^{-1}; the chord is 221±16 µm s^{-1}, which agrees with a value previously reported for the endothelial P_{os}. The error due to the delay imposed by diffusion of sucrose across the unstirred layer is estimated as 10–15% after the initial 15 s, so that for the present transients which last several minutes extrapolation appears permissible. A solution of the diffusion–convection equation $\partial C/\partial t = D\partial^2C/\partial x^2 - Jv\partial C/\partial x$ for the semi-infinte case is $C_{(x,t)} = C_0/2\{\text{erfc}[(x-Jv.t)/2\sqrt{(Dt)}]+\exp(Jv.x/D).\text{erfc}[(x+Jv.t)/2\sqrt{(Dt)}]\}$, which for long times and comparatively small Jv tends to $C = C_0\exp(Jv.x/2D)$. 'Natural convection'[16] in the boundary layer also runs counter to diffusional delays.

These results reveal either a high conductance ($Lp = 114$ µm s^{-1}; steady-state $P_{os} = 115$ µm s^{-1}; P_{os} $(t = 0) = 166$ µm s^{-1}) or a low conductance (steady-state $P_{os} = 26$ µm s^{-1}), depending on the experimental conditions. The numerical values obtained for the high conductance are of a similar order of magnitude, suggesting that both hydraulic and osmotic flows could traverse the same high-conductance pathway. The dependence of steady-state P_{os} on $\Delta\pi$ could be explained by progressive 'recruitment' of the high-conductance pathway at the higher values of $\Delta\pi$. We speculate that a similar mechanism might have a regulatory role in physiological conditions.

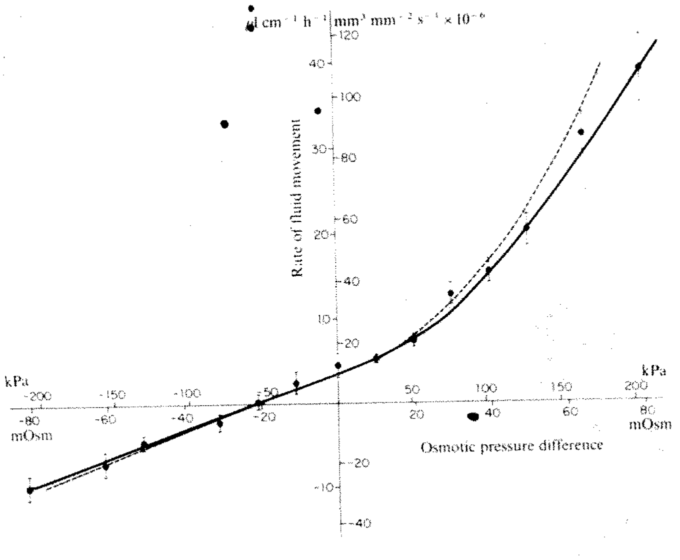

Fig. 3 Rate of transendothelial fluid movement induced by a difference in osmotic pressure. Average and s.e.m. values shown. The solid line was fitted by eye; the dashed line incorporates corrections for solute polarisation (the values for the limiting slopes or apparent P_{os} are 88 ± 6 and 25 ± 3 µm s^{-1} for the first and third quadrants, respectively; correction changes these values to 115 and 26 µm s^{-1}. Direction of fluid movements as above; positive osmotic pressure difference means aqueous hypertonic to stroma, and vice versa. The hydrostatic pressure difference was 10 cm H_2O.

The possibility that the intercellular junctions may be the site of this high-conductance pathway is supported by arguments from two lines of reasoning. In the first, if the junctions are assumed to be water-filled slits which allow laminar flow, one has: $Lp = a^3b/12\,\eta l$, where a is junctional width, b is $\frac{1}{2}$ total cell perimeter/unit area (10^3 cm cm^{-2}), η is viscosity of water (6×10^{-3} P) and l is length of junctions (1 µm). For an Lp of 114 µm s^{-1} (0.8×10^{-11} cm^2 s g^{-1}) the junctional width would be 39 Å. This figure is consistent with anatomical estimates (30–40 Å) and with the fact that horseradish peroxidase (estimated diameter: 45 Å) traverses these junctions[5].

In the second, if the junctions are assumed to be impermeable to water, the osmotic pathway would consist of the basolateral and apical cell membrane in series. If they are assumed uniform, from the P_{os} $(t = 0)$ value, the P_{os} for the cell membranes would be 200 µm s^{-1}. Although P_{os} values as high as this have been reported for erythrocytes[17], in the present framework the value found for the low conductance would be difficult to explain. For instance, a collapse of the intercellular spaces which would exclude the lateral membranes from the osmotic pathway would reduce the endothelial P_{os} to some 100 µm s^{-1}, rather than to the 26 µm s^{-1} found. On the other hand, if the high conductance is attributed to the intercellular junctions, the transition to low conductance can be explained either by flow, osmolarity and time-dependent variations in the apparent reflection coefficient of the junctions for solute, or by an actual change in their conductance. In the extreme in which osmotic flow through the junctions becomes small, the low conductance value found would correspond almost entirely to a P_{os} of 30 µm s^{-1} for the cell membranes. Such a value is of the order of those found for other animal cells[9] such as the "inner membrane' of frog skin epithelium[18] (24 µm s^{-1}), and for some types of lipid bilayers (19 µm s^{-1}) (ref. 19).

In summary, the possibility that the high-conductance pathway corresponds to the intercellular junctions whereas the low

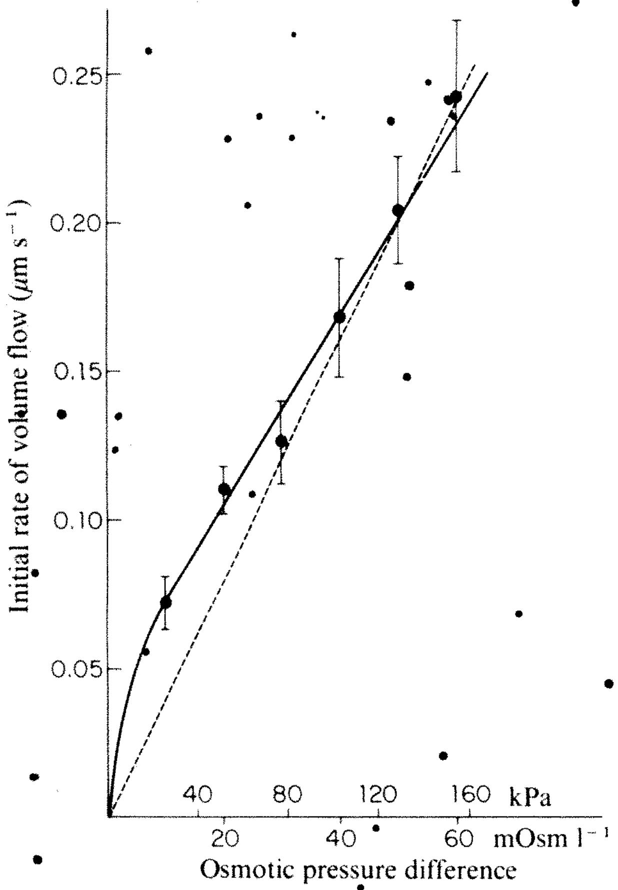

Fig. 4 Initial rates of osmotic flow (extrapolated at $t = 0$) plotted against the driving force. Average and s.e.m. values shown. The solid line was fitted by eye; the dashed line represents the chord. The basal rate of fluid transport superimposed (approx. 0.012 μm s^{-1}) was neglected. The hydrostatic pressure difference was 20 cm H_2O.

conductance reflects properties of the cell membranes seems consistent with the present results. The detailed role of each pathway in the fluid transport mechanism across this and other epithelia remains a central question.

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Experimental model of ulcerative colitis

AN experimental animal model of human ulcerative colitis has long been required, as there is no model with an immunological basis available. We report here the induction of inflammatory ulcerative changes in rat colon mucosa, similar to those of human ulcerative colitis. The lesions were consistently obtained by an experimental procedure that induces specific adult anti-foetal tissue immunoreactivity.

Rat foetal colon implants beneath the renal capsule of syngeneic adult rats developed, by 6 d, acute inflammation and later ulceration of the mucosa morphologically indistinguishable from active ulcerative colitis. The subrenal capsular implantation method for organ growth employed has been used as a model of delayed hypersensitivity of murine small intestinal mucosa in an allogeneic system[1,2]. In the present experiments, the syngeneic implantation permitted survival of the colon implants which, although not rejected, showed continuing immunological inflammatory damage of the mucosa. It is possible that ulcerative colitis and presumably other autoimmune diseases of man may follow or be exacerbated by immunoresponsiveness to foetal antigens, which may re-emerge in adult tissues in certain pathological conditions[3].

The syngeneic rats were the Sprague–Dawley strain maintained in Monash University by brother–sister mating; they accepted tail skin syngeneic grafts but not allografts. Ten male (250 g) and ten female (190 g) rats received sub-renal-capsule implants of descending colon from 16–18-d-old foetal rats from two litters obtained by caesarean section of pregnant females of this inbred strain. Halves of each descending colon were implanted into male and female recipient pairs. A left lumbar approach to the left kidney of anaesthetised recipient rats was used in each case and the pairs of male and female recipients were killed at 48-h intervals, that is up to 20 d. The time scale of changes in the implanted colon was further investigated in five male and five female Sprague–Dawley rats which were killed in pairs 3, 5, 21, 28 and 32 d after implantation of colons from another litter of syngeneic foetal rats.

With the single exception of one rat after 28 d, the implanted syngeneic foetal colon remained structurally and functionally viable as shown by vascularisation by recipient blood vessels and continued production by the mucosa of intestine-specific mucin which stained positively by immunofluorescence with a specific anti-intestine serum[4], and also

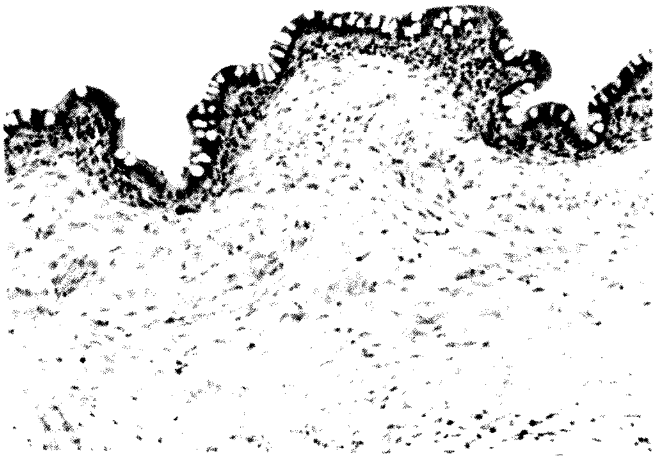

Fig. 1 Rat foetal colon implanted beneath renal capsule of syngeneic adult for 6 d. Note viable mucosa with prominent mucin droplets and a broad band of leukocytic infiltration beneath. (Paraffin section stained with haematoxylin and eosin; × 90.)

with periodic acid-Schiff and alcian blue (pH 2.8). After 6 d the graft (in both male and female recipients) had become infiltrated by lymphocytes and eosinophils (Fig. 1). By 12 d many plasma cells were present and the mucosa was ulcerated with an abundant infiltration by neutrophil granulocytes at the sites of ulceration; the lumen of the graft also contained collections of granulocytes and large quantities of mucin (Fig. 2). Gradually over the succeeding days, the mucosa became depleted of mucin-secreting cells, and by 20 d it was largely destroyed. These changes were found consistently in all the pairs up to d 20. At d 28, the implanted foetal colon in one rat was reduced to a fibrous sac; in the other 28-d rat the implanted colon was well preserved overall, showing as the only pathological change, a microscopic mucosal diverticulum invested by an abundant focal lymphoid reaction. In one of the rats killed on d 32, there was again good preservation of the implant with only microscopic ulceration, in the other 32-d rat, the implant showed advanced changes such as occurred in the 12–21 d experiments.

The sex of the foetal rats donating implants was not determined in the 15 rats from three litters used in the above experiments, but a sample from each rat was implanted into male and female adult pairs. The findings in both male and female pairs of recipients were usually similar and no systematic differences were observed. We concluded that the pathological changes in the colonic implants are not attributable to sex-related antigenicity. To examine the strain specificity of the response, five male and five female Sprague–Dawley rats were given sub-renal-capsule implants of DA rat foetal colon. All of these allogeneic implants were totally destroyed within 10 d of implantation.

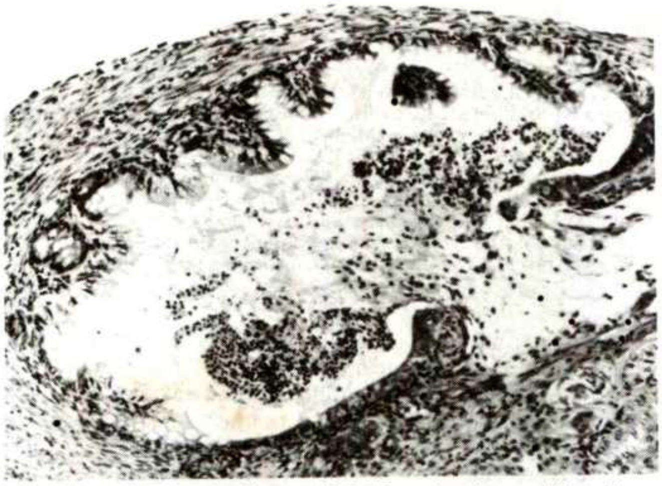

Fig. 2 Foetal colon implant at 12 d. Note ulcer lower right with conspicuous granulocytic exudate extending into lumen filled with mucin. Remaining mucosa looks viable but with less mucin production around the ulcer; it shows conspicuous submucosal leukocytic infiltration. (Paraffin section stained with haematoxylin and eosin; ×90.)

Serum samples from the majority of Sprague–Dawley rats 10 d after implanting syngeneic foetal colon, contained antibody reactive with foetal intestinal mucosa but not adult mucosa, as judged by indirect immunofluorescent staining[5] (Fig. 3). This indicates an immune reaction in the adult rats to foetal antigens deficient in the adult animal. Such a reaction has been demonstrated[6] by inoculating adult rats with syngeneic foetal intestinal material.

The lymphocyte and plasma cell infiltration and the serum antibody activity can be regarded as evidence of an immune response in the recipient to foetal antigens. The inflammatory and subsequent ulcerative reactions can also be attributable to immune rejection of foetal colonic mucosa and experiments to establish this are in progress.

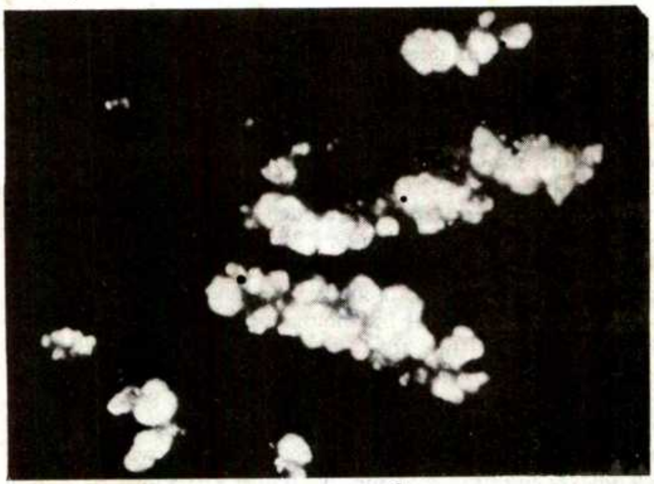

Fig. 3 Immunofluorescent staining of rat foetal colon mucosa by serum from adult rat implanted with foetal colon for 17 d. (Frozen section stained by sandwich immunofluorescence with fluorescein-labelled rabbit anti-rat-globulin, ×110.)

The morphological features of the lesions in the foetal colon implants are similar to those of human ulcerative colitis, which is also believed to have an immunological basis[7]. Patients with this disease have circulating lymphocytes which are cytotoxic to colon epithelial cells, and non-cytotoxic serum antibody directed against cytoplasmic components of colon epithelial cells. This rat system should provide a useful new model for investigating immunologically-determined colonic inflammatory disease. It will be interesting to see if it is possible to stimulate anti-colonic autoimmune activity in the recipient rats.

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K-contractures and membrane potential in mammalian skeletal muscle

A FUNDAMENTAL property of skeletal muscle fibres is the ability to develop tension in response to surface membrane depolarisation. The relation between membrane potential (V_m) and tension has been measured in amphibian muscle using external potassium concentration (K_0) to clamp V_m [1–4]. A K-contracture follows exposure to high K_0 and tension is graded with K_0 between 20 mM ($V_m = -50$ mV) and 50 mM ($V_m = -40$ mV). Human intercostal muscle, however, either does not respond mechanically to K_0 of 80 mM or gives a very small contracture[5]. It is not clear whether this is a result of diffusion delays in the large bundles of fibres or whether there is a basic species difference in excitation–contraction coupling. I report here that, in bundles of fewer than 20 fibres, the relationship between K-contracture tension and K_0 is shifted to higher K_0 in rat muscle because changes in K_0 are less effective in altering V_m.

Adult male rats were killed by ether anaesthesia. The soleus, extensor digitorum longus (EDL) or sternomastoid muscle was isolated; bathed in solution A (Table 1), and bubbled with 95% O_2 and 5% CO_2 during dissection at 20 °C. The sternomastoid has red and white segments and bundles of red or white fibres can be isolated[6]. Bundles were mounted in a rapid flow bath[7] at 37 °C. One tendon was fixed to an RCA valve and isometric tension recorded on a Grass Polygraph pen recorder. Twitch tension was 2.5 and 2.8 kg cm^{-2} in two sternomastoid bundles (cross-sectional areas measured after fixation for electron microscopy). The tetanus to twitch ratio was between 4:1 and 5:1 in whole muscles and small bundles. Similar bundles from toad (*Bufo marinus*) semitendinosus muscles, in amphibian Ringers[7] at 20 °C were also used.

Records from mammalian bundles exposed to 80 mM K_0 are shown in Fig. 1. Neither EDL (Fig. 1*a*) nor white sternomastoid (Fig. 1*c*) gave contractures although twitch tension fell quickly and recovered after the return to 3.5 mM K_0 within 30 min (EDL) to 1 h (white sternomastoid) without potentiation. Contractures from soleus (Fig. 1*b*) and red sternomastoid (Fig. 1*d*) bundles rarely exceeded twitch tension. Recovery of twitch tension in normal solution took 10 min (red sternomastoid) and 30 min (soleus) and was followed by potentiation. In the same system toad semitendinosus bundles gave contractures of four and nine times twitch tension with typical plateau and spontaneous decay phases. Recovery of twitch tension took 3 min in normal solution and was followed by potentiation. Clearly the responses of mammalian and amphibian bundles to 80 mM K_0 are significantly different, and rat mucles have a response similar to that reported for human intercostal muscle.

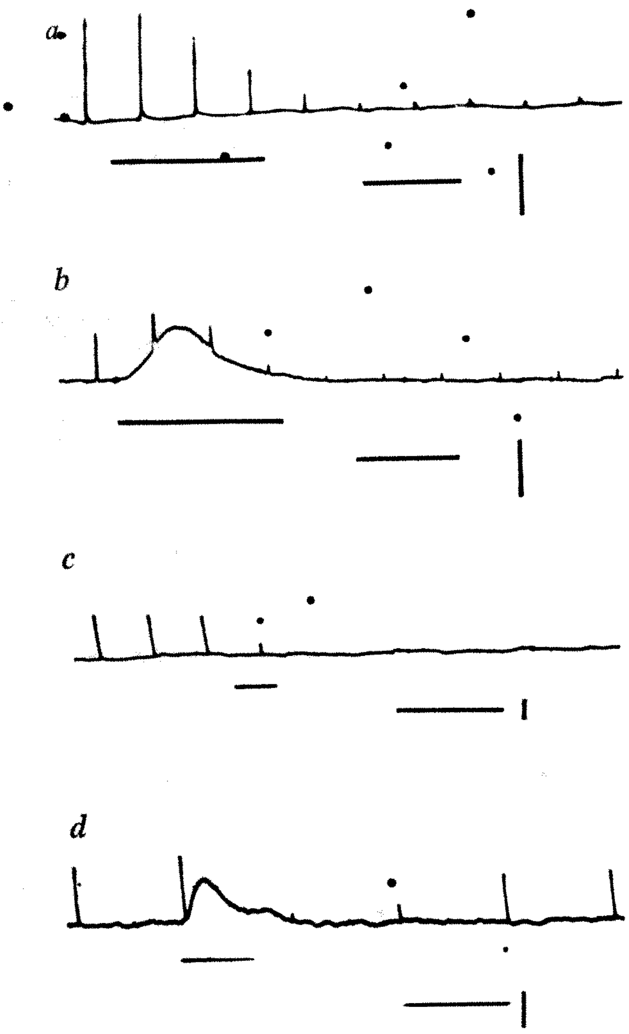

Fig. 1 Tension during exposure to 80 mM K_0 (Solution B, Table 1) for a period indicated by the horizontal bars under each record. *a*, EDL; *b*, soleus; *c*, white sternomastoid; *d*, red sternomastoid. Rapid vertical deflections are twitches (direct electrical stimulation). Scale: tension, vertical bar, 50 mg; time, horizontal bar, 20 s (*a* and *b*) and 60 s (*c* and *d*).

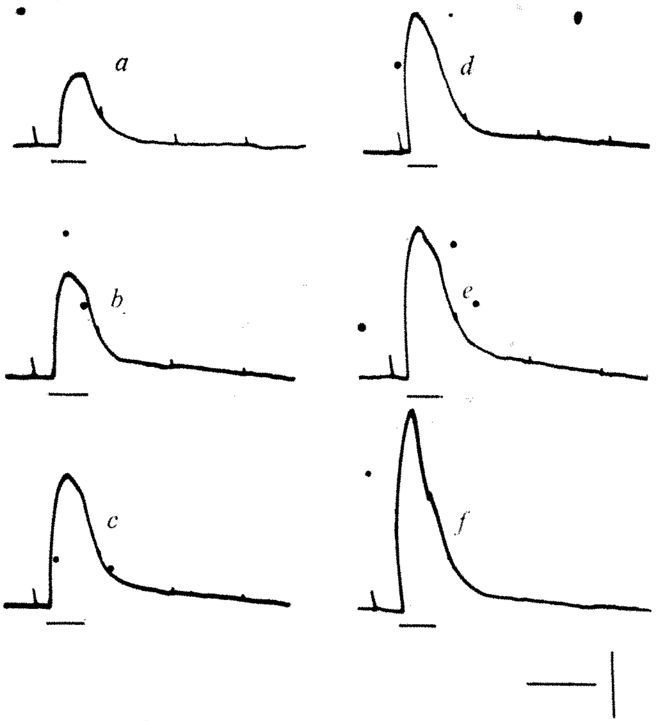

Fig. 2 Contractures from a bundle of red sternomastoid fibres exposed to different K_0 for a period indicated by the horizontal bars under each record. *a* 90 mM K_0; *b* 112 mM K_0; *c* 135 mM K_0; *d* 158 mM K_0; *e* 180 mM K_0; *f* 200 mM K_0. 45 min was allowed between contractures. Test solutions of K_0 up to 180 mM were made with appropriate proportions of solutions C and D (Table 1). Solution E was used for K_0 of 200 mM. The external chloride concentration was kept constant so that the K×Cl product varied. Scale: tension, vertical bar, 100 mg, time, horizontal bar, 60 s.

The responses (Fig. 1) seem to fall into two groups according to fibre type. The EDL contains mostly white fibres[7,8] and its response was similar to that of the white sternomastoid. The soleus, which contains mostly red fibres, responded in a manner similar to that of the red sternomastoid. Comparison of responses with twitch speed gave no consistent relationship since red and white sternomastoid bundles had similar contraction times and were five and ten times faster than the EDL and soleus respectively.

The smaller K-contracture in mammalian muscle was investigated further in red sternomastoid fibres by examining peak contracture tension and V_m when K_0 varied from 3.5 to 200 mM. The solutions were hypertonic for K_0 greater than 120 mM and so test and control solutions of 468 mOsm were used to avoid transient effects of tonicity on tension[10,11]. Twitch tension fell by 80% or more in the hypertonic control solution while tetanic tension fell by 10% so that the tetanus to twitch ratio was 20:1.

Table 1 Solutions used

	Concentrations (mM)*		
Solution	Na	K	Cl
A	165	3.5	174.5
B	88.5	80	175.5
C	206	3.5	216.5
D	28	180	215.5
E	28	200	235
F	28	220	255

*In addition each solution contained glucose 11 mM, Ca 2.5 mM, Mg 1.0 mM, HCO_3 25 mM and HPO_4 3.0 mM.

K_0 up to 200 mM induced no contracture in white sternomastoid bundles. Contracture tension in red sternomastoid bundles increased with K_0 (Fig. 2). Tension in 200 mM K_0 (Fig. 2*f*) was 10 times twitch tension but less than tetanic tension. Greater contracture tension might have been recorded if K_0 above 200 mM had been used. Toad bundles developed maximum tension with 30–60 mM K_0. Higher concentrations (up to 120 mM) increased the rate of tension development, reduced plateau duration and increased the rate of spontaneous relaxation. Similar results have been reported for single frog fibres[1,12,23]. The kinetics of mammalian contractures (Fig. 2) showed a similar dependence on K_0. Tension in high K_0 was shifted to higher K_0 in mammalian muscle. This can be explained by a change either in the relation between tension and V_m or in the relation between V_m and K_0.

V_m was recorded in red sternomastoid fibres using intracellular microelectrodes filled with 3 M KCl and the average resting V_m was -65 ± 1.5 mV. The steady-state V_m (after 3 min.) is plotted against log K_0 in Fig. 3: the line through the points has a slope of 28 mV, less than expected for a potassium electrode (61.5 mV) and less than reported for amphibian fibres (45 mV) with the same concentrations. The hypertonic conditions are not responsible for the observed slope, for points recorded in normal solutions fit the same line. The reduced slope would shift tension to higher K_0 in mammalian muscle.

The time course of depolarisation of red sternomastoid fibres in high K_0 was surprisingly slow, taking 3 min, with a half time of 20–30 s, while repolarisation in 3.5 K_0 took 30 min. The time course was even longer in white sternomastoid fibres. But toad semitendinosus fibres, from bundles of a similar size, took 2 s to depolarise from -80 to -60 mV in 20 mM K_0 with a half time of 4–5 s. It seems unlikely therefore that the time course of V_m in mammalian fibres is an artefact.

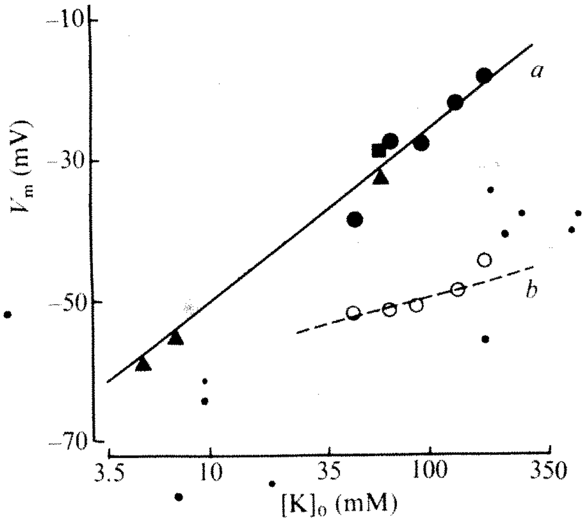

Fig. 3 V_m, in mV, as a function of the logarithm of K_0 in mM. ○, V_m20; ●, steady-state V_m from the same fibres. Each point is the average of three or four fibres and contains the standard errors. Tetrodotoxin (2×10^{-8} g ml^{-1}) prevented action potentials and twitches. Bundles were stretched to 1.8 times rest length to reduce contractures. The fibres were equilibrated in hypertonic solutions for 1 h before the experiment. ■, fibres in isotonic solutions. Curve *a* was drawn through the points by eye and line *b* was drawn through the V_m20 points assuming the relation between V_m20 and log $(K)_0$ is linear.

The relation between tension and K_0 may be shifted to higher K_0 by the reduced rate of change of V_m if depolarised mammalian fibres are subject to contractile inactivation in the same way as amphibian fibres[1,3]. Spontaneous contracture relaxation (Fig. 2) suggests inactivation, so that tension may be inhibited before the steady-state V_m is achieved. Maximum tension was reached within 20 s (Fig. 2) and is therefore a function of V_m 20 s after the solution change (V_m20). V_m20 is plotted against log K_0 in Figure 3 and is less than 50% of the steady-state V_m.

The reduction in K-contracture tension in mammalian muscle is a combined effect of the small change in the steady-state V_m and the slow time course of depolarisation following exposure to high K_0. In 80 mM K_0 mammalian fibres respond to a V_m20 of -51 mV while amphibian fibres respond to a V_m that quickly approaches -30 mV, a potential considerably more positive than the contracture threshold. The relation between tension and V_m in mammalian fibres is shown in Fig. 4. Comparison with data from ref. 1 (see Fig. 4) shows that the true relation between tension and V_m is similar in mammalian and amphibian skeletal muscle.

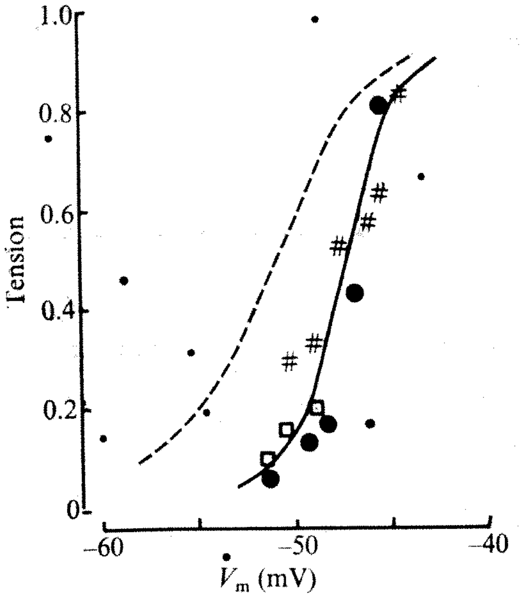

Fig. 4 Tension (in relative units) as a function of V_m in mV. V_m was taken from V_m20 (line *b* in Fig. 3). The symbols are peak tension during K–contractures and records taken from one bundle have the same symbol. The solid line was drawn by eye. The broken line is for amphibian skeletal muscle, ref. 1.

What are the reasons for the slope of 28 mV relating V_m to log K_0 and for the slow time course of V_m changes in sternomastoid fibres? The 28-mV slope (Fig. 3) indicates that another ion contributes to V_m. This is probably not sodium because the resting sodium permeability is low and ouabain (10^{-4}M) does not alter V_m in 60 min. The ion is probably chloride. Concentrations of chloride are not in equilibrium with the resting V_m in several preparations[14–17] and I have found that replacement of external chloride by sulphate increases the average resting V_m in sternomastoid fibres from -64 mV to -76 mV and increases the slope of the line relating V_m to log K_0 to 44 mV. The slow time course of V_m is unlikely to result from diffusion delays for reasons given above. 'Creep' arising from ion depletion in the T tubules[18,19] was recorded and is greater in mammalian fibres than in amphibian fibres with a time constant approximately 10 times longer. Restricted diffusion into the T tubules would increase the time course of changes in V_m.

Thus, bundles of mammalian fibres develop less tension in high K_0 than bundles of amphibian fibres. The true relation between tension and V_m is similar in the two species but the relation between V_m and K_0 for a given change in K_0 is different in two respects; first the change in V_m for a change in K_0 is 40% less in mammalian fibres and second the rate of change of V_m after an increase in K_0 is more than 10 times slower.

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Synthesis of phospholipids and membranes in prebiotic conditions

IT is generally agreed that stable membranes were prerequisite to the assembly of the earliest self-replicating systems[1–4]. Phospholipids, which are ubiquitous in biological membranes and which self-assemble in aqueous environments into stable lipid bilayers and vesicles[4], are obvious candidates for prebiotic membrane components. We report here the abiotic synthesis of various lipids, including membranogenic phospholipids.

Our rationale in establishing simulated primaeval conditions included considerations of possible precursors, energy sources, catalysts and the behaviour and stability of the reactants and products. Derivatives of 1,2-diacylglycerophosphate are common membrane lipids, suggesting fatty acids, glycerol and phosphate as precursors. Heat derived from the Sun was a major primaeval source of energy[5], and recent evidence indicates that average temperate zone surface temperatures may have reached 70 °C before 3×10^9 yr ago[6]. The abiotic synthesis of polypeptides (molecular weight>1,600) at 65 °C, in the absence of water or catalysts, has been detected in amino acid mixtures after incubation times of 40 d (ref. 7); we selected similar conditions for our experiments. Although various clays such as kaolin (an aluminum silicate) have been found to promote condensation reactions in aqueous conditions[8,9], this mineral is rare in Precambrian sediments[10]. However, silicon oxide or silica is present in nearly all rocks[11], and silicate rocks have been shown to promote condensations in certain conditions[12]. We tested the catalytic properties of silicic acid (silica gel H) and kaolin as possible models for natural silica-containing sands and clays. (Since completing this work, we have learned that washed and ignited sea sand promotes polypeptide formation when heated with mixtures of amino acid[13].) Dicyanamide has been widely used as a possible prebiotic condensing agent, and as it has been shown to catalyse glycerol phosphorylation in acid solutions[5], it was included in some experiments. Our general rationale was that tide pools containing dilute solutions of glycerol, phosphate and cyanamide, and having surface films of various hydrocarbon derivatives, could evaporate and produce mixtures of hydrophobic and hydrophilic compounds within a hot sand or clay matrix.

To synthesise neutral lipids, ^{14}C-glycerol and various C12 lipids were mixed in duplicate vessels and the solvent was

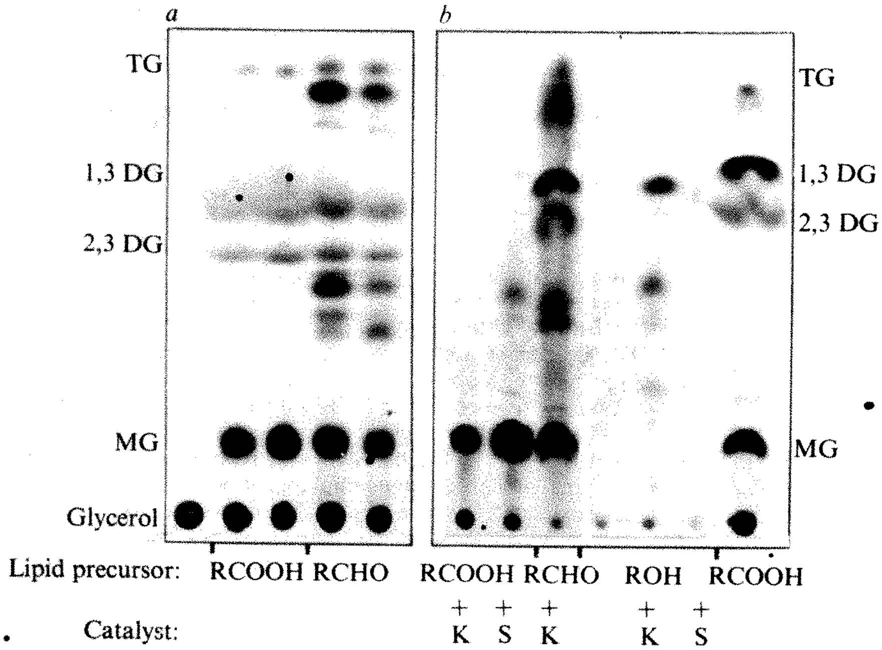

Fig. 1 Autoradiograms of ^{14}C-glycerolipids including mono- (MG), di- (DG), and triglycerides (TG). 50 nmol ^{14}C-glycerol (2×10^6 d.p.m., ICN, Irvine) and 250 nmol C12 acid (RCOOH, Sigma), alcohol (ROH, Sigma), or aldehyde (RCHO, Aldrich) were placed in autoclaved microreaction vessels with loose fitting screw tops. In some cases, 10 mg kaolin (K, J. T. Baker, Phillipsburg) or silica gel H (S, Sigma), prewashed with acetone and chloroform–methanol, was added to the vessel before the removal of the organic solvent by vacuum. Reaction vessels containing aldehyde were sealed individually in small plastic canisters flushed with argon and containing a few $CaCl_2$ pellets. In one experiment using fatty acid and fatty alcohol, open test tubes were used, without desiccant (reactants were suspended in H_2O which was evaporated during the first 2–3 d of incubation). Lipid yield from fatty acid was indistinguishable from the experiment using vacuum-evaporated microreaction vessels. After 1 week at 65 °C, total lipids were extracted in chloroform–methanol or ethyl ether and chromatographed on thin-layer plates of silica gel (F-254; EM Lab., Elmsford) twice in hexane–ethyl ether–acetate (100 : 200 : 1) (Fig. 1*a*), or once in a more complex solvent system[20] followed by the first solvent (Fig. 1*b*). Autoradiography was performed with Kodak RP-Royal X-omat film; exposure times of 3–7 d at −85 °C were followed by development in Diafine. One week exposure revealed 50–100 d.p.m. ^{14}C. Standard lipids were monolaurin (Sigma), dilaurin (Supelco, Bellefonte) and trilaurin (Sigma), run as internal standards in some samples and adjacent to unknowns in some cases. Identification of unknowns is tentative, as it is based solely on TLC R_fs in several solvent systems (some samples were eluted and rechromatographed). Glycerol and lipids were used as received, except the aldehyde, which was purified on silicic acid in hexane and stored under argon.

removed by vacuum or by evaporation during subsequent incubation at 65 °C. Relatively short chain, saturated lipids were used as precursors for glycerolipids because they are fluid at 20–46 °C and are not labile to oxidation within the chain. In some cases, prewashed kaolin or silicic acid was added before the removal of the solvent. After drying, all samples were incubated for 1 week. Lipids were extracted and chromatographed on thin-layer plates of silica gel, placed over X-ray film for autoradiography, and the radioactive spots were scraped for scintillation counting. Typical autoradiographic patterns are shown in Fig. 1. Glycerol incubated alone showed only a single spot at the origin. Monoglyceride was the major reaction product from fatty acid, and was also prominent in the fatty aldehyde samples. Also tentatively identified were 1,3- and 1,2-diglycerides, and small amounts of triglycerides and several unknown lipids, possibly long chain cyclic acetals of glycerol. Total yields of glycerolipids were 3.1% from fatty acid and 7.7% from fatty aldehyde. Fatty alcohol formed little, if any, monoglyceride or other glycerolipid in the absence of added agents. With kaolin, however, detectable amounts of labelled lipids (0.2% yield) were formed.

Figure 2 compares the net yield of labelled lipid in the presence and absence of kaolin and silica gel. Surprisingly, kaolin seems to inhibit the formation of labelled lipids from fatty acid, but has much less effect on the reactivity of glycerol–fatty aldehyde. Silicic acid promotes the formation of monoglycerides from fatty acid (3.8% yield compared to 1.8% yield without catalyst), but inhibits the synthesis of more complex glycerolipids.

As initial experiments suggested that monoglycerides and more complex glycerolipids could have accumulated in evaporated tide pools, we selected an ether monoglyceride (chimyl alcohol) as a precursor for phospholipids. Ether monoglyceride is stable in the conditions used and avoided the requirement for large amounts of glycerol and fatty acid in the samples. Silicic acid, kaolin and dicyanamide were tested as possible condensing agents. Duplicate samples containing ether monoglyceride, dodecanoate and prewashed silicic acid and kaolin were mixed by vortexing in 2 ml H_2O containing phosphate (20 mM) and glycerol (6.5 mM), or sodium dicyanamide (50 mM), or both (Table 1). All tubes were incubated sealed, for 12 h at 65 °C, then all but one pair were allowed to evaporate to dryness in the vented oven. After 2 d the open tubes were completely dry in appearance and, after another 7 d, all tubes were extracted with chloroform–methanol–0.1 M KCl (ref. 14). Polar lipids were precipitated from acetone and chromatographed on thin-layer plates.

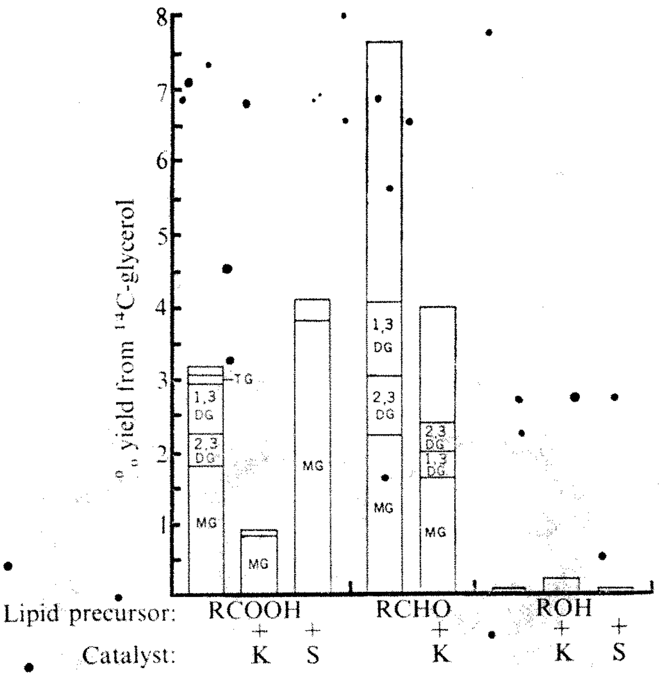

Fig. 2 Quantitation of ^{14}C-glycerolipids from C12 acid (RCOOH), aldehyde (RCHO) and alcohol (ROH). Radioactive lipids detected by autoradiography were scraped from TLC plates into scintillation vials. One ml H_2O was added and after 1 h, 5 ml cocktail ('Tritosol' modified from Fricke[21]) was added. Percentage yield is based on estimated initial 1.8×10^6 c.p.m., though total c.p.m. recovered from samples free of K and S were substantially lower, due to vaporisation of glycerol and adsorption to containment canister when present. RCHO samples contained substantial amounts of apparent triglycerides, but it was not feasible to count these spots separately from large amounts of an unknown lipid running just below. Unlabelled sections of bars in all other cases represent unidentified compounds.

The polar lipid fraction from sample 2 (Table 1) contained phospholipids with R_fs similar to phosphatidic acid (PA), phosphatidyl glycerol (PG), and phosphatidyl glycerophosphate (PGP), and some residual neutral lipids. Total yield of lipid phosphorus was 0.2% of the phosphate in the reaction mixture. Samples 1 and 4 each yielded <0.015% lipid phosphorus, suggesting that dicyanamide and evaporation together enhanced phospholipid synthesis more than tenfold. Several polar lipid-like compounds (ninhydrin negative) were present in sample 3, but no phospholipids were found.

To determine if the partially purifield phospholipids could form membranes, an aliquot from the polar fraction of

Table 1 Synthesis of phospholipid. All samples contained ether monoglyceride (chimyl alcohol, 10 mg, Sigma), dodecanoate (10 mg), prewashed K (50 mg), prewashed S (50 mg), and sodium azide (0.01% initial concentration). When present, phosphate was 20 mM Na_2HPO_4, *p*H 7.8, glycerol was 6.5 mM, and sodium dicyanamide (Aldrich) was 50 mM. Lipids extracted from alkaline samples after addition of 0.1 M KCl (ref. 14) were only partly soluble in chloroform (C) or ethyl ether (E), but readily soluble in C–methanol (M) (2 : 1). Polar lipids were precipitated by adding 10 volumes of acetone to 1 volume of C–E (1 : 1) containing partially solubilised lipid and storing at −20 °C overnight. Insoluble (polar) lipids were collected after centrifugation in a clinical centrifuge at 4 °C. Phospholipid was identified by spray reagent[22] and by the method of Lowry *et al.*[23] after scraping spots from charred preparative plates of silica gel H run in C–M–H_2O (65 : 25 : 4). Compounds similar to PA, PGP and PG were present at greater than 0.065, 0.016, and 0.005 μmol lipid phosphate in the acetone fractions of sample 2. Samples 1 and 4 each contained less than 0.006 μmol total lipid phosphorus.

Sample	Treatment	Phosphate	Glycerol	Dicyanamide	Phospholipid detected
1	Aqueous	+	+	+	Trace
2	Evaporated to dryness	+	+	+	0.2% Yield
3	Evaporated to dryness	—	—	+	None
4	Evaporated to dryness	+	+	—	Trace

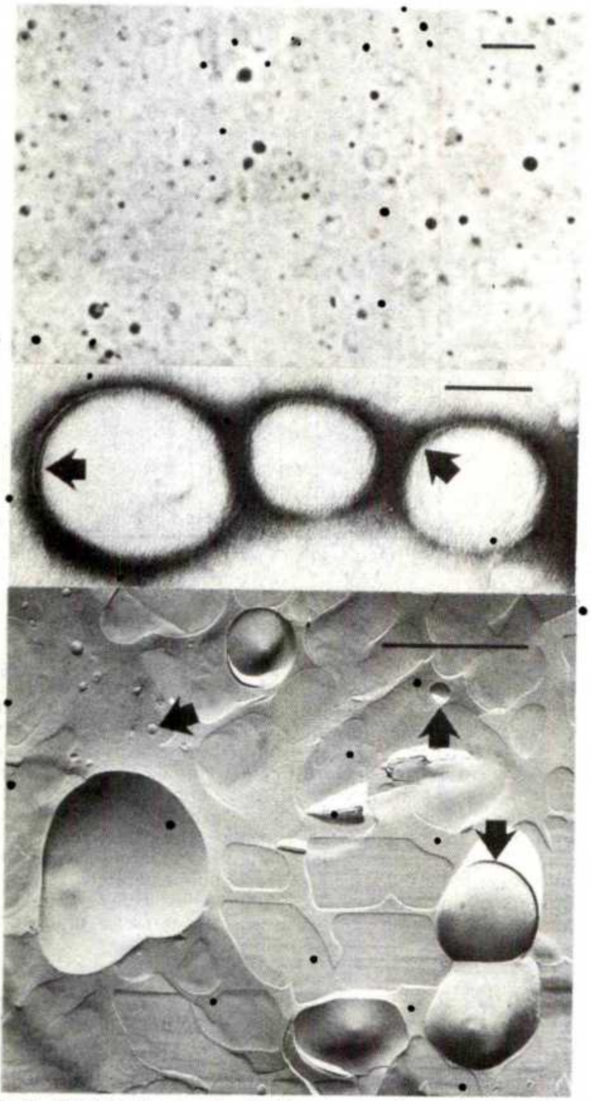

Fig. 3 Vesicles from polar lipid extracts of sample 2. *a*, Phase contrast micrograph (bar is 10 μm) of lipid suspension (1 mg ml^{-1}, made by evaporating solvent from aliquot in small tube, adding 30 mM NaCl, 2mM Na_2HPO_4 (*p*H 7.4), and warming to 50–55 °C for 1 min. *b*, Negative stain electron micrograph (bar is 0.1 μm) of above preparation after probe sonication (Bronwill Biosonik, Will Corp., Rochester, New York) for 10 s at 53 °C. Vesicles were stained with uranyl acetate (1 %) after sample was applied to carbon–formvar coated grid and blotted dry. Note several lamellae (arrows). *c*, Freeze-fracture electron micrograph (bar is 1.0 μm) of lipid suspension (5 mg ml^{-1}) made by warming lipid film as above with the buffer containing 25 % glycerol as a cryoprotectant. Note vesicles of various sizes (arrows).

sample 2 was dried in a small tube. The sample was suspended in 30 mM NaCl, 2 mM Na_2HPO_4 (*p*H 7.4) by warming to 50 °C in a water bath. After a few seconds, a slightly opalescent solution with no visible particles was obtained. Phase contrast microscopy revealed a heterogeneous suspension of apparent vesicles (Fig. 3*a*). Brief sonication with a probe sonicator at 50–55 °C resulted in a more homogeneous population of vesicles (negative stain electron micrograph, Fig. 3*b*). A concentrated suspension, made without sonication in the above buffer containing 25% glycerol, was prepared for freeze-fracture electron microscopy. This technique exposes the hydrophobic plane of lipid bilayer membranes and preserves the structure of lipid–water phases[15]. The presence of lipid vesicles of various sizes in this preparation (Fig. 3*c*) confirmed our light microscope and negative stain electron microscope observations.

This work has implications concerning the possible origin of life on Earth. Although considerable progress has been made in the abiotic synthesis of important biomolecules, including phospholipid precursors, in simulated primaeval conditions[5,16], the only similar studies of prebiotic glycerolipid synthesis are those of Degans *et al.*[8,9], who reported the synthesis of neutral lipids from glycerol and fatty acids in a water–kaolin mixture held at 85 °C for 2 weeks. Neither reagent purity nor control experiments were described, however, and yields were relatively small (about 0.1%). Our results show that both fatty acid and fatty aldehyde react rapidly with glycerol in the absence of catalysts to form the precursors of membrane lipids, and that phospholipids and lipid-membrane vesicles can assemble in possible prebiotic conditions. We suggest that silicates other than kaolin are likely to have promoted such syntheses.

It is significant that phosphatidyl glycerol was apparently formed in our reaction mixture. This lipid, and its aminoacyl derivatives, are prominent in most prokaryotic membranes[17]. Our results show that membrane-forming lipids require only three precursors: glycerol, fatty acid or aldehyde, and phosphate. An alternative and well-studied model system for prebiotic membranes consists of proteinoid 'microspheres', prepared from concentrated solutions of amino acid polymers[18]. There is general agreement, however, that biological membranes require lipid bilayers as barriers to free diffusion of metal ions and substrate molecules. Because our results show that membranogenic lipids are readily produced in simulated prebiotic conditions, we concur with suggestions (Goldacre in ref. 4)[3,4,19] that lipid bilayer membranes could have been present before the accumulation of proteins, polynucleotides and other complex molecules.

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Recommended reading

During the past few years several publishers have produced series of books or pamphlets on specialist topics designed for use on undergraduate courses, particularly those in biological disciplines. Seven reviewers with experience of teaching such courses present here their impressions of the extent to which these series have been successful, in their particular fields, as additional teaching material for students.

THERE was a time when individual authors were able to write encyclopaedic works about their subject, and they frequently conveyed their information with an air of bland authority. In botany, names such as Sachs, Strasburger, Kerner and Oliver will generate waves of nostalgia among the older generation of plant scientists. In the early decades of this century, undergraduate courses could be taught using just one or two major texts as background reading. Modern single-author textbooks are usually far more restricted in the topics they cover and rarely claim to summarise all knowledge concerning that subject, more often they are content to illustrate the basic principles of their subject by reference to specific examples.

There have been some attempts at the encyclopaedic approach even in recent years, but such efforts have not been greeted with enthusiasm by the academic world. In these days of intense specialisation the experts in each of the topics covered are sure to find fault with the section dealing with their pet topic in any such book. And, since no student will read a textbook from cover to cover, if they cannot have certain sections specifically recommended, they are unlikely to pick up the book at all.

Multi-author books, sometimes with one or two editors involved in their production, represent one answer to the publisher's dilemma. Such books are often very successful, but may suffer from an uneven style and quality. An alternative is the series of monographs, each written by a specialist in the field, and each relatively independent of the others This overcomes inconsistencies in style and permits a relatively low cost for each volume. This approach has been taken by many publishers both at research and at undergraduate levels. Pioneers in the undergraduate biology sphere have been Edward Arnold (London), publishers of the *Studies in Biology* series (hardback and paperback editions).

Overall, this series has probably been more successful than any other which has been attempted. The secret of its success lies in the publisher's skill in the selection of topics and determination of length (and, thereby, cost). For example, the first issue of the series was *Ecological Energetics* by John Phillipson (£2.25, 95 pence) and this has become a classic of ecological literature within its ten years of life. The topic is sufficiently well defined to be outlined in about fifty pages and has been admirably condensed by the author. Similarly successful issues have been *Plants and Water* by J. Sutcliffe (£1.30 paperback), *Population Dynamics* by M E. Solomon (£2.20; £1.10), and *Photosynthesis* by D. O. Hall and K. K. Rao (second edition, March 1976; £2.80; £1.40). It is difficult to avoid the temptation to produce broad topic books—such as *Biology of Pollution* by Kenneth Mellanby (£1.75; 75 pence) and *Microecology* by J. L Cloudsley-Thompson (paperback £1.20)—which may do well on the bookstalls because of their general appeal, but which try to cover too great a scope to be valuable as course texts. The opposite end of the spectrum is also to be found in the series, particularly in the later issues (are the publishers running short of ideas or authors?). This extreme is represented by topics which are concise to the point of having limited appeal, such as *The Biology of Eucalypts* by L. D. Pryor (£3.20, £1.60) and *The Secretion of Milk* by Ben Mepham (£2 80; £1.40).

Plant Taxonomy by V. H. Heywood (£2.60; £1 30) has now entered a second edition (1976) and forms a useful introduction to experimental taxonomy. It contains some interesting chapters on chemical and numerical approaches to the subject which help to dispel the old-fashioned image of this subject. *Genetics and Adaptation* by E. B. Ford (£2.60, £1.30) is a new issue which is bound to sell well. The basic genetics are dealt with in two short chapters and the remainder of the book has a distinctly ecological flavour, dealing with such subjects as selection, melanism and isolation. This should be widely recommended as a background to a variety of biological courses, all first-year biology students should read it.

Tissue culture is now a frequently used technique with which most advanced level school biologists will have had some contact. *Plant Tissue Culture* by D. N. Butcher and D. S. Ingram (£3; £1.50) provides a guide to the implications and applications of the technique and also supplies the practical information needed for setting up simple tissue culture experiments in the laboratory.

Two more specialised studies which have been issued during the past year are *Phytochrome and Plant Growth* by R. E. Kendrick and B Frankland (£3; £1 50) and *Lichens as Pollution Monitors* by D. L. Hawksworth and F. Rose (£2.80, £1 40). The former is a timely review of a fascinating and fast developing area of plant physiology, and once again it includes simple laboratory experiments. The second book is really an expansion of the authors' paper in *Nature* (**227**, 145; 1970) It makes interesting reading, but is rather specialised for this series and suffers from the disadvantage that the taxonomy of lichens is sufficiently difficult to put the application of this technique for pollution detection out of the reach of the average undergraduate. Species descriptions are included but a simple, illustrated key to the more important indicator species would have made this a much more useful book.

The *Tertiary Level Biology* series (hardback and paperback), published by Blackie (Glasgow and London), also aims at the undergraduate, but has a more advanced approach, thus making it of value to postgraduates too. *Water and Plants* by H. Meidner and D. W. Sheriff (£5.50; £2.90) is the latest issue in this series, and begins by describing the properties of water and the concept of water potential. In a logical order it then considers water vapour and transpiration, water transport in the plant, and the plant–soil relationship. This is certainly not an introductory book and the undergraduate would need to be weaned on a lower level text, such as the Edward Arnold study by J. Sutcliffe. It does, however, provide a full, thermodynamic and mathematical basis for understanding the current state of the study of plant–water relations.

Hutchinson (London) have also begun a series of introductory studies in biology. The third and latest title in this series is *Microbial Plant Pathology* by P. J. Whitney (£4.95; £2.95). This is a well-produced and readable book which assumes no previous knowledge of the subject. It begins with an account of disease transmission which concentrates on crop diseases, and which underlines the economic importance of plant pathology. It then considers infection, defence mechanisms and pathogen-induced reaction in the host species. It is a clear and concise introduction to the subject and is good value for its price.

One of the latest issues in the *Oxford Biology Readers* series (Oxford University London) also deals with plant pathology and is entitled *The Control of Plant Disease* (30 pence), by B. E. J. Wheeler (who, incidentally, is also author of *Diseases in Crops* in the Edward Arnold series). The idea behind the *Oxford Biology Readers* is brevity and, consequently, low price. These aims are undeniably achieved, but at the expense of depth. Usually they are about 15–30 pages long, and this kind of treatment demands very careful selection of material Some subjects are more suitable for a brief survey than others. Plant pathology cannot be covered adequately in 15 pages although Wheeler does achieve an interesting introduction to the problem of disease control. The topic chosen for an issue in this series must, therefore, be relatively narrow in scope. For example, in the issue entitled *Stomata* (60 pence), Heath admirably attains a detailed analysis of the physiology and mode of action of these organs.

Oxford Biology Readers are performing a very different function from the other monographs reviewed here in that they have such limited coverage within each issue. Because of this they do not generally form a basis for a specific undergraduate course but provide supplementary information on certain topics within such a course This must limit their sales potential among undergraduates despite the high quailty of some of these booklets

Having examined several of the biological series currently being served up for undergraduate consumption, one cannot help being impressed by the Edward Arnold's *Studies in Biology* These appear to have struck the best length of treatment and selection of topics to meet the widest student demand. The publishers should be congratulated on anticipating so accurately the growing demand for this type of publication. **Peter D. Moore**

Peter Moore is Lecturer in the Department of Plant Sciences, King's College, University of London, UK.

BIOLOGY has become such a diverse subject, and one in which there are so many rapidly-developing areas, that the single, comprehensive textbook covering the whole field has become largely obsolete. A new development has been the production of series of smaller, cheaper books, to which new, up-to-date titles on specific topics can be added as they become appropriate. Such series have the advantage that lecturers and teachers can selectively stock their libraries with material that is directly relevant to their particular courses without having to pay for additional material they do not require.

The *Oxford Biology Readers* series (Oxford University: London) are essentially a series of essays on a variety of biological topics—some very specific, others more general—written by authors who are pre-eminent in their particular fields. Each provides a concise and authoritative introduction to its subject They are well produced with clear photographs and excellent diagrams and they include fairly comprehensive suggestions for further reading They are excellent value and provide an invaluable source of basic information for students at advanced school and undergraduate levels.

In *Primate Locomotion* (45 pence) Professor J. R. Napier describes the variety of locomotor patterns found in primates and discusses how these have evolved in relation to the habitats in which primates live and the various types of food that they eat. He also discusses the rather scanty evidence relating to the locomotion of fossil primates and the special skeletal adaptations for bipedalism shown by man.

Insect Flight (45 pence) by Professor J. W. S. Pringle deals with many aspects of a complex subject in a remarkably clear and concise way. He discusses the different mechanisms found in insects, the aerodynamics of flight, the physiology of insect flight muscles and the control and coordination of flying This *Reader*, which is illustrated by exceptionally clear diagrams, can only provide a rather cursory examination of such topics, but perhaps its principal value is that it encourages the reader to pursue the subject further.

The Echinoderms are a group of animals that differ from other Phyla in so many ways that there is a tendency to regard them as somehow being off the main stream of evolution Professor D. Nichols, in *The Uniqueness of Echinoderms* (45 pence) sets out to convince the reader not only that they have a fascination in their own right but that (because of their abundance in the marine environment and the fact that, through the echinoderm larva, they may be the ancestors of the chordates) they are entitled to more attention from zoologists. His enthusiasm for these animals is evident in this lively and interesting little book.

In *Evolution Studied by Observation and Experiment* (45 pence), Professor E. B. Ford discusses the contribution to our understanding of evolution made by the study of ecological genetics, a field in which he has been a major influence. He shows how the study of various characters under natural conditions has revealed that selection pressures favouring advantageous characters are much more powerful than Darwin and other early evolutionary theorists had believed was possible and that natural selection can bring about adaptive changes very much more quickly than was previously supposed His evidence is drawn from studies by himself and others, including those on colour patterns in butterflies, shell colouration in snails and the human blood groups

During the development of the science of animal behaviour certain theoretical concepts have been developed and have subsequently been the focus of considerable debate and disagreement. Robert and Joan Hinde discuss two such concepts in *Instinct and Intelligence* (45 pence) Using a wealth of examples they show how instinct has become a somewhat disreputable concept, whereas intelligence now has a precise scientific meaning, more restricted than its everyday usage. They argue strongly against using either concept as an explanation for behavioural phenomena and suggest that they are important as ideas that point to problems and to areas of investigation, which should ultimately make them redundant

The story of the evolution of the birds from the reptiles through the fossil *Archeopteryx*, has been told many times. In *The Evolution of Flying and Flightless Birds* (45 pence), Sir Gavin de Beer provides a concise and well-illustrated discussion of this topic, together with the evolution of the ratites, a group which, because of their flightlessness and geographical distribution, have exercised the minds of evolutionary theorists for many years. Since de Beer's death in 1972 there have been some interesting developments in this field, notably Ostrom's re-analysis of *Archeopteryx*'s wing and Cracraft's theory that the ratites are descended from a single flightless ancestor which was dispersed by continental drift.

All these *Readers* can be highly recommended. They provide, in a concise and clear way, an invaluable source of information which can provide a sound basis for further study.

The philosophy behind Arnold's *Studies in Biology* series (London) is an essentially sound one. The series provides a medium by which biologists can be kept up to date with developments in specific areas of biology. They are reasonably priced and are an ideal way by which individuals, schools, colleges and universities can supplement their libraries in particular fields.

Small rodents and insectivores have provided much of the data that form the basis of population and community ecology. In *The Ecology of Small Mammals* (£1.90; 95 pence) Dr M. S. Delany strikes a nice balance between discussing them as animals of interest in their own right because of their abundance, economic importance and role in the terrestrial ecosystem, and showing how they have contributed to general ecological theory. He covers a great deal of material, deliberately widening his subject matter to include species from as far afield as East Africa, America and the Far East. He does not attempt a comprehensive review of this material but aims to highlight the areas in which new developments are taking place.

The book begins with a discussion of some of the basic methods of trapping small mammals and of estimating their ages, home ranges and population densities. It then considers the life histories and demographic patterns found among these animals in different habitat types around the world. This is followed by a chapter on populations and the way they are affected by seasonal changes, food supply and predation. The final chapter deals with relationships between small mammal species and the way communities of species are related to different habitats.

Dr Delany's book is clearly and concisely written, well illustrated and fully referenced Perhaps its greatest virtue is that it leaves the reader with the motivation to read further on the subject.

The student of zoology is generally brought up with the notion that animals are anatomically symmetrical, and great stress is laid on such characters as radial and bilateral symmetry as fundamental features of different animal types. In *Animal Asymmetry* (£2.60, £1.30) Dr A. C. Neville points out that asymmetrical organisation is commonly found right across the Animal Kingdom. For each example he shows how asymmetry is adaptive in the life of the animal. Some insects have left and right wings of slightly different shapes so that they can be folded one over the other. Owls have differently shaped left and right ear apertures that enable them to locate sounds with great accuracy. Such asymmetries raise interesting questions in developmental biology and Dr Neville discusses briefly how they may arise during embryonic development in molluscs, insects and other animals. He also points out that asymmetry is a property of organic molecules and of the carbon atom itself.

Dr R. F. Chapman's book, *A Biology of Locusts* (£2.80; £1.40), should prove invaluable to any school or university laboratory that keeps a colony of locusts. It deals with the basic biology of these insects, their life history, feeding behaviour, flight and migration. The book also discusses the ecology of locusts in the wild, particularly from the point of view of their impact as serious agricultural pests. Finally, he discusses the various techniques that have been used to control locusts and also those that are now being developed. This is a very useful book because it contains, in a very readable and clearly illustrated form, virtually all that one could want to know about animals that are used very widely by students at all levels.

The aim of the series, *Topics in Biological Sciences*, published by Hutchinson (London), is to emphasise the social and economic importance of a number of biological topics. *The Biology of Insects* (£4.95; £2.95), by C P. Friedlander, begins with an account of the structure, physiology and natural history of insects, along the lines that one finds in many biological textbooks. This is followed by a chapter on their behaviour and ecology and, finally, a chapter on the economic importance of insects and the methods used to control them. So wide is the scope of this book that its coverage of any one topic is of necessity rather superficial, and it is difficult to see that it represents an advance on existing entomology texts. *The Biology of Locusts*, however, in the *Studies in Biology* series, which considers only one species of insect, brings out much more successfully the way that an understanding of an insect pest's biology can contribute to its effective control.

Also in this series, *Freshwater Biology* (£4.95; £2.95), by L. G. Willoughby, similarly attempts to cover a very wide field. It deals successively, as most books on freshwater biology do, with the physical and chemical properties of water, with freshwater plants including algae, and with a number of aquatic invertebrates and fishes. There is a brief chapter on features peculiar to running water habitats, and a longer one on decomposition cycles and nutrient recirculation. The chapters on social and economic aspects, such as water supplies, sewage treatment and pollution are very brief and serve only to outline these important topics. This book suffers, like its companion volume, from trying to cover too much material in too small a book. The attempt to combine basic biology with social aspects is not very successful; the latter seem to have been added almost as an afterthought. These books are not cheap and certainly not as good value for money as the *Studies in Biology* series.

These three series provide an interesting contrast. The authors of the *Oxford Biology Readers* are for the most part senior, distinguished biologists, and they have written with the authority one would expect on what are generally traditional biological topics. The *Studies in Biology* series are mostly written by a younger generation of authors, and their titles and style reflect a rather more progressive approach to biology. Both series can be highly recommended; they are cheap, well illustrated and well written. There is perhaps a risk that the *Oxford Biology Readers* will be used merely as 'essay cribs' by students; the *Studies* are more likely to stimulate an enquiring approach in their readers. The Hutchinson series has not been nearly as successful as the other two. Although well illustrated, they attempt to cover too wide a field in each volume, and their price brings them into competition with more thorough and complete textbooks, with which they do not compare very well.

T. R. Halliday

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THE emergence of membranes as a major growth area in biological science has naturally provoked a proliferation of student texts in this area. These supplement the accounts to be found in general textbooks of biochemistry and cell biology and would therefore be expected to provide the integration and advanced information that these texts omit, but without too much repetition of readily available foundation material.

An attractive book, appropriate for students, is Weissman and Claiborne's *Cell Membranes: Biochemistry, Cell Biology and Pathology* (HP Publishing: New York, 1975, £12.00), but the price means that students are likely to read it only in a library It is well written by eminent authors, but the multiauthor format sometimes leads to repetition (for example, Rasmussen and Goldberg, Pappas and Loewenstein) and to an imbalance between the relative importance of a topic and the space allowed to it.

A student wanting his own membrane text then has a choice. Either he can get one of several small books that aim at a complete basic coverage of membranes, such as Saier and Stiles, Harrison and Lunt or a competitor (Quinn, *Molecular Biology of Cell Membranes*, Macmillan, 1976, £8.45; £3.85; Finean *et al.*, *Membranes and their Cellular Functions*, Blackwell, 1974, £2.80; Parsons (ed.), *Biological Membranes*, Oxford University, 1975, £5.00) or he can select his information piecemeal from series publications such as Oxford University Press' *Biology Readers* (London) or Chapman and Hall's *Outline Studies in Biology* (London).

Of the single texts, M. H. Saier and C. O. Stiles' *Molecular Dynamics in Biological Membranes* in the *Heidelberg Science Library* (Springer: Berlin and New York, DM 13.80; $5.70) is most biologically oriented (despite its misleading title). The authors have a firm, maybe correct, unitary belief that membranes are all similar and that function invariably depends on structural fluidity; this sometimes leads to unsupported statements such as "(Intercellular) junction formation is undoubtedly dependent on membrane fluidity". Sometimes, they lose sight, however, of their unitary philosophy, as when sensory chemoreception (for example, taste) and hormonal chemoreception (for example, insulin receptor) appear in different chapters. These problems, and the limited and personal choice of topics discussed, makes the book unsatisfactory as a central student text. It does, however, include useful accounts of a number of membrane-mediated processes not normally treated in small texts (for example, bacterial chemotaxis, membrane fusion, and so on), provided the reader does not expect attempts at molecular explanations.

By contrast, R. Harrison and G. G. Lunt, in *Biological Membranes Their Structure and Function* in the *Tertiary Level Biology* series (Blackie Glasgow and London, £7.80 hardback; £3.90 paperback), have aimed at a systematic and balanced text with membrane chemistry as its dominant theme The result is good but patchy They are at their best when discussing "Membrane carbohydrate and cell surface specificity" and in a valuable and concise section on physical methods used in membrane studies More than half of the remaining space is allotted to membrane, morphology, composition, isolation and structure, with a lot of space occupied by electron micrographs of doubtful educational value and by basic lipid chemistry (including three illustrations of the same glycerophospholipid structures) This overindulgence restricts the space available to membrane functions (transport, secretion, turnover, enzymes, receptors, and so on) even though they have equal billing in the title.

Membranes feature in the *Outline Studies in Biology* series (£1.30 each) in the recent books by A. M. Malkinson and by M. F. Greaves, in M Davies' *Functions of Biological Membranes* (better titled *Membrane Transport*) and in a promised *Membrane Assembly*. Malkinson's *Hormone Action* presents twin discussions on hormone actions mediated through cell surface receptors and through intracellular receptors. This contrast is useful, and the material presented is up-to-date Only about one third of the whole, however, relates to membranes Greave's *Cellular Recognition* is an excellent text that draws together diverse phenomena (hormones, neurotransmitters, the immune system, tissue differentiation, sex in yeasts, and so on) and discusses them in terms of the reception and interpretation of environmental information at the cell surface. It is logical and well organised, with substantial sections on membrane structure, receptors, and mechanisms of stimulus–response coupling. A major virtue of series publications is that it can allow an articulate specialist to pass on his fascination with such a problem. But the series format also has drawbacks the high cost of collecting the books needed to achieve a final patchy coverage, duplication of fundamental information on membrane structure, and sometimes poor editing and presentation.

Two *Oxford Biology Readers* fall into the membrane category, and are straightforward biochemical introductions to their topics. J A. Lucy in *The Plasma Membrane* (45 pence), has packed a lot of basic information on membrane structure, turnover, fusion and transport (illustrated diagrammatically as an unlikely rotating macromolecule) into his 16 pages. Hormones are left to P. J. Randle and R. M Denton's *Hormones and Cell Metabolism* (45 pence), a balanced and appropriate short account of metabolic control. This, however, has little on membranes other than control of adenylate cyclase, and is marred by inconsistency of units (insulin) and nomenclature (phosphorylase), repetition of diagrams and some almost illegible yellow-on-white printing. Other useful membrane-related titles in this series are *Lysosomes* (A. C. Allison). *The Golgi Apparatus* (G. M. W. Cook), *Mitochondria* (J. B Chappell and S. C Rees) and *Differentiation in Higher Plants* (D. H. Northcote).

If I could select only one item from this collection it would undoubtedly be Greave's *Cellular Recognition*: it wins both on scientific quality and value for money, even though it explores only one area of membrane function.

Bob Michell

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"OF making many books there is no end . . . Fear God, for this is the whole duty of man" Statements like this frequently explode from scientists drowned in a suffocating morass of reading—even though their theological intent may be different to the original Old Testament context. Nevertheless it could be an apposite comment about the eight books under review: all come from long and continuing series, four from Chapman and Hall's *Outline Studies in Biology* which includes 22 titles published since 1973 (London, distributed in USA by Halsted/Wiley: New York; three from Arnold's *Studies in Biology*—71 published since 1966 (London); and No. 72 in Oxford University Press'. *Biology Readers* series, which began publication in in 1971 (London)). All the series state that they are trying to fill gaps left by standard textbooks, either at the interface between traditional disciplines or in rapidly developing topics where textbooks are quickly out-of-date.

The Oxford series is the slightest in format, designed so that each one "will fit conveniently in a file among the students own notes" The series is specifically aimed at sixth-formers (school children at advanced level), students in Colleges of Education and Polytechnics, and first-year undergraduates. Each volume (really pamphlet—they have about 14 pages) covers a specific limited subject in reasonable depth and with good illustrations. J. L. Jinks's *Cytoplasmic Inheritance* (45 pence) is only one of 19 in the series devoted to genetics and evolution; like its companion volumes it has no bibliography but a list of "further reading", which includes five books and five reviews or key papers.

The other two series are directed at the second- to third-year university level, although they are in general free from jargon and thus potentially valuable to sixth-formers and their ilk. Chapman and Hall's *Outline Studies* (paperback editions only) cover subjects which may be expected to form a normal university course of 5–10 lectures. Arnold's *Studies* (hardback and paperback editions), on the other hand, tend to deal with topics that do not fall neatly into usual teaching slots, and for that reason seem to be more stimulating. For example, Bernard John's *Population Cytogenetics* (£3; £1.50) is concerned with both animal and plant chromosomes; with population phenomena as well as straight cell biology, and with the quantitative effects of chromosome substitution. This enables the author to point out that knowledge is lacking about population cytodynamics and the effects of chromosome rearrangements on the genetical structure of populations—fairly complicated concepts which emerge naturally because of the interdisciplinary treatment of the subject. In contrast, D M. Moore's *Plant Cytogenetics* (£1.30) and L M Cook's *Population Genetics* (£1 30) in the *Outline Studies* series, although both are excellent in their own ways (Cook in particular weaves mathematical formulation with natural history in a refreshingly non-intimidating manner), are almost inevitably more pedestrian. They will be useful for supplementing courses where no teacher is available for the topics considered (or alternatively, for providing unversed teachers with prepared teaching material).

One disadvantage of the lecture-course approach in a book is that it exposes gaps in treatment which would (or should) not appear in a comprehensive textbook and which would probably be filled by other teaching in a complete school or university course. For example, R A. Woods in *Biochemical Genetics* (*Outline Studies*, £1.30) gives a useful summary of gene action and its control in microorganisms, but does little more than mention higher organisms. He justifies this limitation by claiming that "the information they [microorganisms] have yielded is 'cleaner' and more suited to an outline text" True, but this means that the title of the book is misleading. Again in the same series, A. McDermott gives a full introduction to human chromosomes and their variation in the *Cytogenetics of Man and Other Animals* (£1.30) (a topic which is only touched on in general biology or genetics texts), but *other animals* are only referred to in passing, largely in relation to human studies.

The *Studies in Biology* series is sponsored by the Institute of Biology, and this academic input may explain their imaginative choice of topics. One criticism that has been levelled at the series in the past is the relative lack of references in many of the volumes. In fact the three volumes from the series under review seem to contain enough key references for practical purposes. The *Outline Studies* series is variable· two of the present books have fairly extensive bibliographies, the other two merely a selection of "further reading".

Two other recent and relevant titles in the *Studies in Biology* series are *Genetics and Adaptation* characterised by E B. Ford's usual lucid and idiosyncratic style; and *Population Dynamics* by M. E. Solomon, a new edition of a useful book first published in 1969 and now fairly extensively revised

One great advantage of limited topic books is that leaders of research in particular fields can write on their own topic: four of the eight under review are written by authors who have previously published substantial works on the same subject (Cook, Ford, Jinks and John) The *Studies* and *Outline Studies* series both work out at 2 pence per page in paperback; this is quite good value for money nowadays. It is difficult, however, not to hark back to the Penguin *New Biology* series which lasted from 1946 to 1960 and filled the gaps in textbooks so well at the time. But perhaps biology as well as publishing has changed . . . **R. J. Berry**

R. J Berry is Professor of Genetics in the University of London, UK.

FEW books pack so many lessons for the plant physiological world as the slim volume on *Translocation in Plants* by Michael Richardson, No. 10 in Arnold's *Studies in Biology* series (£1.90; 95 pence). The book—now in its second edition and proclaimed an Open University Set Book —has already served many generations since first published in 1968. Why its popularity? It takes the form of six chapters with well defined subsections, almost suggesting it began life as a revamped set of lecture notes. Perhaps this explains its sales success, but also its failings?

Probably the most glaring error is the use of a water hammer (pp18–19) to measure the cohesive properties of xylem sap. Nowadays students see more mass spectrometers than Berthelot tubes or water hammers, and few would have sufficient knowledge to question explanations given Dixon made the water hammer (1914) to illustrate cohesive attraction between water and glass in the presence of gas near vacuum. The experiment determining the limiting cohesive tension of water was carried out by Berthelot (1850) in a tube filled completely with water: both systems were described clearly by Dixon.

Phloem research has advanced rapidly in the past decade. Broadly the position is one in which the model proposed by Hartig and expounded more precisely by Münch (1930) has become established and received widespread acceptance. For centuries it was obvious that channels for dry matter translocation existed in bark of plant stems. After the discovery of sieve tubes the main question outstanding was why phloem sap should not flow through these pipe-like cells, carrying dry matter in solution to swell storage organs, ranging from the humble spud to the massive fruits of palms.

The main reason sieve tubes might not function like pipes, translocating the sugary sap, arose from the fact that at frequent intervals sieve tubes are occluded by sieve plates, the holes in which seemed to be plugged in both light and electron microscope studies. This observation gave rise to some alternative hypotheses and "the great phloem controversy". Most alternatives are faithfully reproduced and even extended in the new edition of the book. Yet considerable strides have been made in phloem physiology from studies of plants which, on wounding, exude phloem sap, as might be expected by Münch's hypothesis. Virtually none of this work has found its way into the new edition. There is some justification to withold some of the detailed electron microscopy which has contributed to the widely held view that sieve plates serve a protective function, sealing rapidly when the system is injured. But the omission of this aspect so completely cannot be excused, because it is so central to the theme of the book. Nor is it legitimate to call aphid stylet sap "uncontaminated" (p36) on account of saliva injection effects (see, for example, *Biology of Aphids* by A. F. G. Dixon, *Studies in Biology* No. 44; 75 pence).

Another *experimenta crucis* which has contributed to the phloem controversy is the alleged demonstration of bidirectional transport in phloem. Certainly no sieve tube could, if it functioned as a pipe, conduct simultaneously in opposite directions at rates exceeding those of diffusion. But the experiment chosen to illustrate this, by Palmquist (1939), is dangerously misleading, as is obvious to anyone who has carried out even limited research on the system. The problem is that xylem pipes, containing sap under tension, lie in close proximity to the highly pressurised sieve tubes being investigated. Abraided leaves can rapidly suck solutions into xylem tubes producing an illusion of bidirectional transport. Much better research (for example, Turgeon and Webb, 1973) would have been desirable alternative material. Similarly much of the earlier work could have been omitted without loss. Sadly the five new pages and seven new paragraphs are largely (especially with hindsight) irrelevant.

The success of this book is a disturbing reflection on both scientific and publishing fraternities. If a better alternative of comparable cost existed, I hope students would have been guided to choose it in preference: such a volume is clearly lacking. If errors have escaped criticism so long, does this imply the capacity to publish extends beyond a proper degree of rigorous criticism? It is indeed regrettable that this book mars the high standards of the series as a whole. **John A. Milburn**

John A. Milburn is Senior Lecturer in Botany at the University of Glasgow, UK.

In the past two years, three publishers have made additions, in the field of muscle biology, to their (continuing) series of books designed for late school or undergraduate consumption. Oxford University Press (London), in their *Biology Readers* series, have produced a 16-page pamphlet entitled *The Contractile Behaviour of Mammalian Muscle* (30 pence) by A J. Buller; Arnold (London) have persuaded D. R. Wilkie to write a second edition (hard and paperback) of his book on *Muscle* (£2.50; £1.25) in their *Studies in Biology* series; and Blackie (Glasgow and London) have produced a specialised text on *Visceral Muscle· Its Structure and Function*, (£5.10) by H. Huddart and S. Hunt.

Muscles, according to Professor Buller and Wilkie and Doctors Huddart and Hunt, respectively, are more important in getting animals about than brains, are the means by which we act on our environment and move objects as well as ourselves; are a tissue and therefore by definition composed of cells These paraphrased quotations from the first lines of the three books are characteristic and exemplify their approaches.

The books vary enormously in length and in their scope and coverage. Professor Buller concludes all he has to say in 16 succinct pages, of which 5 are taken up by illustrations Wilkie covers his subject in 67 more sparsely illustrated pages, whereas Huddart and Hunt pack their comprehensive but more specialised subject matter into 165 The shortest work concentrates almost exclusively on isometric and isotonic contractions of fast and slow twitch skeletal muscles, having briefly introduced the subject by way of the ultrastructure and the nervous architecture of muscle. Professor Wilkie, as becomes a pupil of A V. Hill, does not neglect the thermodynamics of muscle energetics, but also introduces the subject by means of the ultrastructure and chemical composition of muscles In his book, he gives an overview, taking his examples from all sorts of muscle and from many species He describes the common experimental methods used in examining muscles, pointing to their shortcomings. He explains the results obtained in terms of the known structure. He leads the reader rather gently through a clarification of the electrical phenomena in quiescent and stimulated muscle and illuminates the chemical changes that result in contraction Finally, he brings us back to everyday experiences by describing how muscles maintain tone and enable us to take exercise.

The largest, although still slim volume, has a wider target for a narrower topic. It claims to be of interest to students of physiology, medicine, biology and pharmacology and deals specifically with *Visceral Muscle* It is the most conventionally organised of the three; once again, the contents run from microscopic structure through innervation to mechanical activity, but conclude with the protein structure and the molecular basis of contraction Although some of the chapters are of course specifically oriented to visceral muscle, it is surprising how often comparisons are made with experimental work in skeletal and cardiac muscle.

Clearly, here is a triad of books that can be used quite differently, yet they are linked by a unity, since much of the material is common to the three. Wilkie's book stands out above others in the way that it simplifies complex ideas and yet has more than enough meat for any hungry student. Anyone remotely interested in muscle—and who among biologists is not—can—and one might almost say should—read it. The result will be not only an increase in knowledge and understanding, but an experience of an outstanding example of written communication. In addition, the author is a realist who has the interest of students at heart; he does not give the usual unmanageable list for further reading, but confines himself to a few seminal works.

Buller's contribution is more a pamphlet than a book; it probably contains all that a student need retain about the fundamentals of muscular contraction The question arises: is he more likely to retain nearly all of this or only a quarter of the slightly longer text? The answer must depend on the assessment of the teacher and the character of the student. It would be reasonable for any biologist or medical student to read a mere 11 pages, but how many have the time or the inclination to read ten times that many on a single subject? The beauty of this pamphlet is that it is short, lucid and expert, yet it does not have unrealistic expectations of the reader.

What then of the last book? It is not for every student; its primary use might be for reference, for each chapter is almost complete in itself, containing at least two key references. In addition, there are some 150 further references, and above all it is the only one of the three with an index.

It seems that these three complementary works offer three solutions to the problem of the modern science student—an elegantly produced evening's reading for tomorrow's seminar, a readable text from which something will be remembered, or the small reference for a narrow subject to be dipped into as required. You pays your money (0 30; £1.25 or £5.10) and you takes your choice.

Rainer Goldsmith

Rainer Goldsmith is Professor of Physiology at Chelsea College, University of London, UK.

Most immunologists are preoccupied with problems pertaining to mice or men, but many are now showing an increasing interest in the immunological responses of other species in order to discover evolutionary trends in recognition of foreigness, structure of antibody molecules and mechanisms of protective immunity.

In *Comparative Immunobiology* (£6 60, £3.30), in Blackie's *Tertiary Level Biology* series, Margaret Manning and Rodney Turner are the first authors to write a textbook of suitable size and clarity for students of immunobiology Indeed, this book can be recommended to anyone seeking an introduction to comparative immunology Other books have been and are being written on this subject but great credit must go to these authors for providing a book which will obviously remain a basic textbook on the subject for many years to come

Those immunologists already involved with the vagaries and complexities of immunological responses in different species will admire the layout of the book. First, there is a 28-page introduction on the basic tenets of immunology, largely derived from work on mammals and birds. There follows an ambitious description of immunological responses as we climb the 'evolutionary tree' from sponges to fishes and terrestial animals Each chapter is introduced by an accurate phylogenetic tree Phyla which have been sufficiently studied by immunologists are lucidly described in clearly defined sections headed 'phagocytosis', 'transplantation reactions', 'humoral reaction and factors' and 'lymphoid tissues'

One of the most intriguing aspects of invertebrate immunology is how animals with no lymphocytes can recognise foreign species. This is treated adequately here, but new theories on recognition now appear monthly

The bursa of Fabricius is wisely described as being unique to birds (p149), and this should be a warning to those immunologists still hunting for a 'bursal equivalent' in mammals

This is a concise book, small in appearance but large in its breadth of treatment and factual content. All immunologists should find time to read it **J. B. Solomon**

J B. Solomon is Senior Lecturer in the Iammunology Unit of the Department of Bacteriology, University of Aberdeen, UK.

Oxford Books for Students

New and forthcoming

Foundations of Mathematics

Ian Stewart and David Tall

This is a book for readers in transition from 'school mathematics' to the fully fledged type of thinking used by professional mathematicians. It should prove useful to first-year students in universities, polytechnics, and colleges, and to sixth-formers contemplating further study £6 paper covers £2.95

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D. W. Jordan and Peter Smith

This textbook in the qualitative theory of nonlinear differential equations is for students in mathematics or in mathematics-orientated combinations and is directed towards practical applications. It treats recent developments and subjects such as singular perturbations and the phenomena associated with forced nonlinear equations that have not been readily accessible before at this level. £11 paper covers £5 50 *Oxford Applied Mathematics and Computing Science Series*

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B. J. Wood and D. G. Fraser

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An Introduction to Human Evolution, Variation, Growth and Ecology

G. A. Harrison, J. S. Weiner
J. M. Tanner and N. A. Barnicot

This textbook has now been revised to include the many recent advances in the fields it covers, especially those in our knowledge of primate and human evolution, genetic variation at the molecular level, and the nature of growth and adaptation processes. There is also a completely new chapter on primate behaviour and the origins of human society. Second edition illustrated £10 paper covers £5.50

Oceanography

Contemporary Readings in Ocean Sciences

Edited by R. Gordon Pirie

First published in 1973, this collection is intended as a supplementary reader for first- and second-year undergraduates in ocean sciences In this second edition the articles are generally briefer, more elementary, and more popular; thirty-one of the forty-six selections are new. Second edition paper covers £6.75

Kinetics of Chemical and Enzyme Catalyzed Reactions

Dennis Piszkiewicz

This volume is intended to serve several functions. It is meant as a means of teaching kinetics to advanced undergraduate and graduate students. It presupposes a background in organic chemistry and some knowledge of calculus, while a familiarity with biochemistry and chemistry would be useful, though not essential. It is also intended as a primer for reading papers in the literature dealing with kinetics. Paper covers £4.75

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Edited by J. L. Knill

This series of essays provides an introductory survey of applied geology for the more advanced undergraduate or the postgraduate student, those who are teaching geology, and planners concerned with geological resources. The contributors, writing from extensive experience, have provided examples of the application of geology in practice and the roles played by geologists in industry. £10

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by **E. Miller**, *University of Southampton.*

Ageing is rightly a topic of increasing concern and research interest. The growing proportion of the elderly in the population raises many important issues for society, and presents some problems. Dementia is one of the commonest and yet most difficult disorders found in older people, and the basic aim of this book is to present the psychological aspects of dementia.
The author reviews what is known about the psychological changes produced by dementia and discusses the practical contributions that psychology can make in dealing with the demented individual.

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Embarking on biochemistry

WHEN, until recently, I lectured to large groups of science or medical students embarking on a course of biochemistry, I took along to the first lecture about 15 different texts. I tried to explain the merits of the various books in terms of the widely varying attitudes of the students After the lecture many students would stand perplexed and some would even express their feelings to plead that they be told what to buy I understood their viewpoint but I think, as teachers, we must resist the students' plea The truth is, of course, that biochemistry is blessed with a wealth of excellent texts and the best-buy for any particular student is seldom an easy decision The problem is made more difficult in the knowledge that unless a student buys a textbook by half-way through the first term he is unlikely to do so at all. In other words he has often to make his choice before he has much idea as to his interest in the subject.

The two texts under review are both excellent in their way. A L Lehninger's *Biochemistry The Molecular Basis of Cell Structure and Function* (Worth. New York, second edition, $17.50) is now clearly a classic and is extremely popular with students both of science and medicine. It is also comparatively cheap in terms of length and the excellence of the printing *The Biochemistry of the Tissues* (Wiley London and New York, second edition, hardback £14 50, $30, paperback £6 25, $13.50) by P Banks, W. Bartley and L M. Birt is a more modest effort, based on their lectures to medical students at Sheffield University

A continual problem in the teaching of biochemistry is to keep the picture of the subject as a whole before the student Too often the older courses appeared fragmentary and the student lost the unity of the subject while they delved into the minutae of the various aspects Lehninger keeps the story going by starting with a set of organising principles and continually referring back to them as he deals with various subjects The story holds together very well and this is undoubtedly a main attraction for the students The Sheffield authors had a particular group of students in mind and have tried to hold attention through a consideration of the biochemistry of the various tissues The student has to be prepared for this approach, however, so that the earlier chapters are a somewhat skimped traditional text

In some ways *Biochemistry* by L Stryer (Freeman San Francisco), has much to offer medical students In this beautifully produced book reference is continually made to the relevance of biochemistry to medicine and physiology Thus there is an excellent chapter on sickle-cell anaemia Indeed there have been attempts to organise a course of biochemistry on a selection of such diseases One thinks of *Biochemistry A Case-Oriented Approach* (Mosby Missouri) by Montgomery *et al*, but again there are snags, for the student must have a proper groundwork before he is ready for this kind of treatment

Another unifying approach that is now often used in first year courses is to develop the biochemistry from an elementary study of cell biology. This is probably better than concentrating on the tissues. So far, however, I have not seen a textbook based on this kind of course

In summary, both books reviewed have their strong points and certainly the latter part of the Sheffield book would prove a useful adjunct to the cheaper and much more comprehensive Lehninger text **P. N. Campbell**

P N Campbell is Director of the Courtauld Institute, Middlesex Hospital Medical School, London, UK.

Biochemical tools

Physical Biochemistry Applications to Biochemistry and Molecular Biology. By D Freifelder Pp. x+570 (Freeman San Francisco, 1976) Hardcover £12.40; softcover £8 00

A THOROUGH understanding of the physicochemical tools used by the modern biochemist is essential to their intelligent use It is unfortunately a common experience of many, if not all, teachers of physical biochemistry, however, that one of the major obstacles in the path of a diligent student is the labour necessary to wade through the often complex and seemingly endless theoretical and mathematical derivations which represent the standard approach to the subject One solution to this dilemma is to choose a limited number of techniques and to develop fundamental equations clearly from first principles with appropriate simplifications Such an approach is justified by the quantitative nature of many of the techniques and has been used successfully by K E Van Holde (also entitled *Physical Biochemistry*, Prentice-Hall)

An alternative and in some respects radical solution is to adopt an almost completely non-mathematical approach Professor Freifelder has done just this with the single aim of enabling "the student to understand and read the current literature" Equations cannot be avoided, of course, but although their derivations are, the physical principles, the assumptions behind the derivations and the conditions which must be satisfied for the equations to hold are clearly defined. *Physical Biochemistry* is therefore an exceptional book in that it neither requires nor contains a knowledge of basic thermodynamics which inevitably characterises other comparable texts. For this alone many students will be thankful

Throughout the book, Professor Freifelder's overriding concern is to stimulate the students' interest in the techniques of physical biochemistry, and if the avoidance of mathematical treatments is one cornerstone of his strategy, then the demonstration of the usefulness of the techniques in solving biological problems is the other Indeed, the outstanding feature of his book is the wealth of biological examples illustrated. The majority of these are drawn from the molecular biology of bacteriophages and the physical biochemistry of DNA and viruses, reflecting Professor Freifelder's research interests and making a welcome change from the more conventional protein examples

The subject matter is generally comprehensive, comprising an extensive section on direct observations by light and electron microscopy, general laboratory methods, separation and identification of materials, hydrodynamic methods including an especially well-written chapter on sedimentation, and spectroscopic methods including a useful introduction to nuclear magnetic resonance. The qualitative approach seems nevertheless to have led to some curious omissions for a text on physical biochemistry Thus all scattering techniques are absent In addition there is no treatment of multiple, small molecule binding Surprisingly, a section on immunological methods is included

This book is properly written for final-year undergraduate and first-year graduate students Many of them will appreciate its non-mathematical approach from which there are undoubtedly great advantages of motivation to be gained. Ultimately, however, the student who finally arrives at the practical application of a technique for his own research purpose can only benefit from a thorough acquaintance with the underlying theoretical and mathematical analysis This book will surely engender in the student sufficient enthusiasm to make the necessary effort **G. L. Kellett**

G L Kellett is Lecturer in Biochemistry at the University of York UK

Endocrinology of steroid hormones

Molecular Endocrinology of the Steroid Hormones By D. Schulster, S. Burstein and B. A Cooke. Pp.xv+321. (Wiley. London and New York; 1976) Cloth £9.75, $20, paper £4.75, $10.

THIS textbook on molecular endocrinology of the steroid hormones is an attempt to focus attention on the mechanism of action of steroid hormones. Its aim is to provide broad coverage with the minimum of fine detail in four main sections—methods, the endocrine glands, regulation of biosynthesis, and mode of action of steroid hormones.

The book is not intended to be a reference text for research workers, but is an account of steroid hormone biosynthesis and mode of action aimed at science students perhaps majoring in biochemistry, or at medical students There is no doubt that a science student with a reasonable background in chemistry and biochemistry could read this text with ease

The methodology of steroids is briefly reviewed with a fairly cursory treatment of radioimmunoassays. It might have been appropriate to have given this aspect of methodology a more extensive treatment. If the text is to be used by preclinical medical students then it is likely that most of the steroid hormone assays which they will request as practising doctors will be carried out using this method A detailed account of the technique including its shortcomings might have been to the reader's advantage

Biosynthesis of steroid hormones is presented in a fashion which will be acceptable to undergraduate science or medical students. Short chapters deal with the structure and function of the pituitary, hypothalamus, adrenal cortex, ovary, testes and the feto-placental unit. These chapters each contain a brief account of the structure and ultrastructure of the endocrine organ, its blood supply and its relationship to other tissues in the body. This section will be extremely useful to students from the physical sciences who wish to grapple with the concepts of endocrinology.

The third section of the book, which is concerned with the regulation of steroid hormone biosynthesis, contains three chapters, the largest being the control of corticoid synthesis by ACTH Problems associated with the acceptance of the usual dogma on the mode of action of ACTH are critically reviewed in this chapter. This is followed by a brief treatment of aldosterone synthesis and a short chapter on the control of androgen and oestrogen secretion by FSH and LH. In view of the considerable interest at the present time on the control of FSH and LH secretion, I would have preferred to have seen a more extensive treatment of the subject. For example, organic chemists and other nonbiological scientists in the pharmaceutical industry involved in work associated with contraceptive preparations could profitably read all of this book, but this section, which would be of great interest to them, is barely seven pages in length.

The production of even a short textbook involves a considerable gestation period The last section dealing with the mode of action of oestrogens, progestins and androgens covers topics which are currently under active investigation Most of the references contained in this section are to papers published pre-1975; because much work has emerged in 1975 and 1976, the medical or science students reading these chapters will be obliged to update the information. The basic concepts of the mode of action of steroid hormones, the localisation of the receptors and the possible nuclear events are constantly undergoing refinement and revision These chapters are superficial, but they will whet the appetite of the student, and the enthusiast will go on to read beyond this text.

It is regrettable that the mode of action of aldosterone does not receive a more detailed treatment. This is one of the few examples in steroid endocrinology where the investigator can in fact obtain a fairly precise physiological indicator of the action of the hormone in an *in vitro* system

This treatment of the endocrinology of steroid hormones will be welcomed by many teachers. The textbook will be valuable to students who are reading biochemistry either for a general degree or for an honours degree, and many of the problems associated with the interpretation of the mode of action of steroid hormones are highlighted Since the subject is so wide the treatment is superficial in parts; but it is always interesting, readable and relatively free from errors. The book can be recommended as a straightforward, clear account of the concepts of molecular endocrinology of steroid hormones in the mid-1970s

G. S. Boyd

G S. Boyd is Chairman of the Department of Biochemistry at the University of Edinburgh, UK, and Honorary Director of an MRC group on sterol metabolism.

Introducing prostaglandins

Prostaglandins An Introduction to their Biochemistry, Physiology and Pharmacology By P B Curtis-Prior Pp xi+158 (North-Holland Amsterdam, New York and Oxford; 1976) Dfl 42; $16 25.

THIS volume is intended as an introduction to prostaglandins for those "currently uninitiated in the subject" The author begins the main text by describing the nomenclature and biosynthesis of prostaglandins. I found the latter section rather misleading in parts—for example, the transformation of endoperoxides to prostaglandins is depicted (wrongly) as a reversible reaction Sections covering prostaglandin catabolism, inhibitors of biosynthesis and receptor antagonists, are followed by a chapter, entitled "Prostaglandin-like substances" (mainly polypeptides), which I felt did not really belong in the book at all The remainder of the text largely comprises an account of the effects of prostaglandins in physiological and pathological processes, although little was said about their role in the control of parturition, or platelet aggregation—surely two areas where the case for prostaglandin involvement is stronger than most? The final chapter deals rather perfunctorily with the important topic of biosynthesis of thromboxanes and other non-prostaglandin products of arachidonate

The text is produced by a process of direct photographic reproduction of the typescript, and the difficulties this causes in the arrangement of the figures give this (otherwise well produced book) a slightly unbalanced look. I found the quality of the artwork rather variable, mainly because half of the figures are reproduced from other sources, a practice which leads to trouble in the final chapter where the wrong structure of thromboxane A_2 is given.

This book is easy to read, but I found the chapters rather superficial and had the impression several times that the author was a little out of touch with the mainstream of prostaglandin research There is, however, no "primer" on prostaglandins to which the "uninitiated" can refer, and no doubt many will be glad to find this information collected in a basic text The book contains adequate references for further study

R. J. Flower

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Enzymes and cellular metabolism

The Enzyme Molecule. By W. Ferdinand. Pp. xvi+289. (Wiley: London and New York, 1976.) Cloth £9.50, $20; paper £4.50, $10.

THE study of enzymes is often regarded as being an end in itself rather than being an essential step towards an understanding of the way in which the cell functions and regulates itself as a biochemical entity. This book makes a valuable contribution to setting the study of enzymes in the wider context of cellular metabolism. The book, which is intended for undergraduate students, requires little initial biochemical knowledge from the reader, although some elementary mathematical ability is assumed.

The topics of simple thermodynamics, single-substrate enzyme kinetics, enzyme purification and protein structure and chemistry are dealt with in some detail in the earlier chapters and an appendix of the book. These chapters lay a solid foundation for the discussion of enzymes which occupies the remaining two thirds of the book. A chapter on enzyme structure and function covers the measurement of ligand binding, determination of residues at the active site, conformational changes in proteins and mechanisms of enzyme catalysis. This last section provides a clear and readable account of transition-state theory which should provide an excellent introduction to those wishing to take the subject further. The author has chosen to consider a single enzyme, ribonuclease, as an example of the chemical mechanism of enzyme action, and the account presented provides a good summary of the evidence which has led to current theories of its catalytic mechanism.

The effects of inhibitors, activators, *p*H and temperature on enzyme activity are covered in a single chapter occupying some fifty pages, which also includes a treatment of the kinetics of enzymes using two substrates. The attempt to cover such a wide range of topics in a relatively short space has led to a rather uneven treatment. The effects of *p*H and temperature on enzyme activity are given rather brief and superficial treatments, whereas the discussion of the Wong and Hanes method for deriving kinetic equations is excellent. In the consideration of the kinetic mechanisms of two-substrate enzymes the author's prejudices do not always coincide with those of the reviewer and generally this section concentrates upon nomenclature to too great an extent without considering the underlying causes of the observed kinetic effects. Cooperativity and allostery are considered in a separate chapter which makes a clear distinction between these two phenomena and discusses the major models of cooperativity and the kinetic predictions that they make. Aspartate transcarbamoylase is chosen as an example of an allosteric enzyme for detailed discussion and the author provides a lucid account of a somewhat confusing situation.

The first chapter of this book considers the role of enzymes within the cell and this topic is returned to in the final two chapters which discuss the role of enzymes in the control of metabolism. One of these chapters provides a somewhat brief outline of the factors affecting the control of enzyme concentration in the cell, whereas the other is an account of the kinetics of the regulation of metabolic pathways. This chapter provides an excellent account of how the enzyme kinetic theory considered earlier can be applied to the regulation of metabolic pathways within the cell. It should be required reading for all those who believe that the study of enzyme kinetics is an end in itself.

An important omission from this book is that there is no treatment of the kinetics of the transient phases of enzyme reactions. The author has obviously had to be selective in his choice of topics in order to deal with the study of enzymes in a relatively short space, but the lack of any treatment of this topic restricts the value of the book as a general introductory text on enzymology. Shortage of space has also lead to a somewhat cryptic presentation of some topics such as X-ray crystallography and experimental methods for studying ligand binding. In addition the author occasionally makes categorical statements that should require qualification For example, it is asserted that the initial rate of enzyme-catalysed reactions is always proportional to enzyme concentration whereas there are in fact a number of exceptions to this and workers should be encouraged to check for such linearity rather than just assuming it.

Despite the above reservations this book provides a good introduction to the study of enzymes and should prove valuable to many students, particularly in showing how this topic relates to the wider fields of biochemistry and cell biology.

K. F. Tipton

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Differing approaches to genetics

SINCE a knowledge of genetics is now considered an important ingredient of many undergraduate courses, as widely disparate as molecular biology and sociology, very different approaches to the subject are to be expected. This is reflected in several undergraduate texts which have appeared in the past year.

M. I. Lerner and W J. Libby's *Heredity, Evolution and Society* (Freeman San Francisco, second edition, $13 95; £8.80) is concerned more with the social consequences of genetics, although it would be unfair to suggest that the authors have neglected the scientific basis of the subject. The emphasis throughout is on the relationship between genetics and evolution, particularly human evolution. Yet though largely, but not exclusively, concerned with human genetics the text is not over-burdened with obscure clinical references which often reduce the appeal of such texts to science students. The standard arrangement of most student textbooks of genetics is not followed For example, chromosome structure and function are not dealt with as such but are included in chapters on gene interaction, sex and mutation. An attractive feature of the book is the use of inserts, called 'boxes', in which are segregated most of the descriptive, biographical and tabular material This is a clearly written text which is eminently readable.

Many biologists are daunted by genetics because of the emphasis on statistics D. T Suzuki and A. J. F. Griffiths recognise this problem, and their book, *An Introduction to Genetic Analysis* (Freeman San Francisco, $13 95) ". . . attempts to teach the reader how *to do* genetics". This is therefore not strictly a textbook, for it is not just concerned with concepts but is more a guide as to how to analyse genetic data. The approach is essentially quantitative, although a level of mathematical sophistication no greater than simple algebra is required It is clearly meant to be a didactic text, extensive use being made of a question-and-answer format, problem-solving exercises (a separate book of answers is available), and the dispersal within the text of so-called "messages" which summarise important information. There are also sequences of diagrams illustrating mitosis, meiosis, DNA replication and translation which can be animated by letting the pages flip over This latter device is not really very satisfactory and seems out of place in a University text Quantitative problems in all aspects of genetics are considered including Mendelian segregation, linear and circular linkage analysis (but not lod score methods), cytogenetic mechanisms and mutation. There is also a chapter on population genetics. Perhaps it is in this latter field that mathematical analysis has been most important, yet this is given relatively little attention The book is wide ranging in the material chosen to illustrate problems in genetic analysis, but it is difficult to see how it will survive competition with more conventional texts.

N. V Rothwell's book *Understanding Genetics* (Williams and Wilkins Baltimore, $14.95), is perhaps the most conventional of these texts. It deals in the usual manner with the mechanisms of gene action, cytogenetics, various aspects of biochemical genetics and population genetics. The molecular basis of inheritance is dealt with very well indeed but rather oddly is relegated to the latter half of the book. This text provides a straight-forward account of genetic mechanisms illustrated by examples from widely related organisms. The illustrations are simple straight-forward line drawings which are very attractively presented. Each chapter concludes with some well chosen references, although very few of these are more recent than 1970 Apart from the chapter on probability there are no student exercises.

W. Hexter and H T Jost's *The Science of Genetics* (Prentice-Hall Englewood Cliffs, New Jersey, £11 90, $18 95) is also an introductory text designed primarily for biology students, but here the authors have tended to use a historical approach in order to emphasise the reasoning which initiated critical studies and lead to important conclusions. The hope is that the book will have a two-fold purpose. " . to introduce the student to the essential facts of genetics and to introduce the student to the methods of science". In general the authors have been successful. Again, illustrative examples are widely chosen from both the plant and animal kingdoms. Of student texts which deal primarily with the science of genetics, this is perhaps the most readable and exciting, probably because of the emphasis on the evolution of concepts and ideas. It also becomes quite clear where there are still some major gaps in our knowledge and understanding, as in the case of chromosome structure The book is enhanced by simple straight-forward line drawings and with references to key papers. Each chapter concludes with a number of exercises but no answers are provided.

These four books therefore reflect very different approaches to genetics For those whose main interest lies in human problems and the social consequences of genetics, Lerner and Libby's book can be unreservedly recommended For those who want a more standard undergraduate text of general genetics, Hexter and Jost's book is perhaps the best.

Alan E. H. Emery

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Abundant facts of immunology

Basic and Clinical Immunology. Edited by H. H. Fudenberg, D. P. Stites, J. L. Caldwell and J V. Wells Pp. 653. (Lange Medical Publications Los Altos, California, 1976) $12.50.

THIS large book is good value for money. Its intent is to act as a source from which the enquiring mind may obtain the abundant facts of immunology. There are impurities in this spring of knowledge some of which should disappear in the promised biennial revisions. The editors solicit our specific advice on this point.

The tone of the book is authoritarian and the referencing is inadequate to check the origins of many of the statements made This may be in line with the current teaching of medical students but it is irritating for the more curious reader The indexing is capricious, which is probably inevitable in a book with forty chapters written by many different people. The line drawings are first class but the reproductions of photographs are in the main of indifferent quality.

The first half of the book deals with the basic aspects of immunology starting with a delightful chapter by Grabar on the historical background The short chronology on important achievements in immunology, which terminates this particular piece, ends at 1959 at a time when many might think immunology was just beginning to gather steam.

The second half of the book starts with a summary of the methods in common use in immunodiagnosis and concludes with a series of chapters on clinical immunology.

The book is fascinating and reminiscent of Florey's texts on general pathology My reservations simply concern the difficulty of teaching rapidly changing subjects without perpetuating too many errors **A. J. S. Davies**

A. J S. Davies is Professor of Immunobiology in the University of London, working at the Chester Beatty Research Institute, London, UK.

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Developing biologists

The Developmental Biology of Plants and Animals, edited by C. F Graham and P. F Wareing (Blackwell Scientific Oxford and London, £7 75; Saunders Philadelphia), is unusually courageous for a textbook in treating development as a biological problem, not simply as zoology or botany. This approach has clear gains (for example, cell interactions and control of differentiation) but also losses a selection of topics that "have a bearing on general themes of plant *and* animal development" (my italics) inevitably excludes many of relevance to only one or the other. On the animal side, the book has nothing on extraembryonic membranes and, more seriously, next to nothing on cell movements.

The book is in six parts, most with an introductory chapter and short conclusion by the editors. Individual chapters are by 20 different authors, mainly writing from British laboratories (except J. T. Bonner from the USA). Integration is achieved by extensive cross-referencing. Figures are used sparingly, giving us a lot of text for our money. The book is advanced, not introductory: most chapters assume basic background, and some use an eclectic range of examples and terminology that would soon baffle a beginner.

In a book of this scope, an individual is bound to have both praise and grumbles over facts or emphasis. Many chapters seem to me admirable critical summaries of our present knowledge—those on animal cell junctions and the control of gene expression are especially clear. On the other hand, the emphasis of the chapter on "self-assembly" seems misplaced. There is lots of physical chemistry, but very little on regulation, surely the central issue for developmental biologists. "Pattern formation in animal embryos" is not for the unwary, reading simply for information: it suggests short-range inductions rather than gradients or positional information as the basic mechanism—a stimulating article, belonging more in a journal than a textbook. The section on environmental effects (rarely mentioned in accounts of animal development) disappoints me. Although most work has been on model animals in laboratories, emphasising the constancy of developmental processes, information is now appearing on ecological factors (for example, on the timing of amphibian metamorphosis), but none of this is mentioned.

Some small points: a student ignorant of *Ascaris* might get the impression that chromosome diminution never occurs (p302). Is vertebrate limb elongation "entirely due to addition of tissue at the tip" (p180)? Why no mention of Roth's (1973) hypothesis as a mechanism of cell interactions (chapter 3 1)? And two irritations: tubulin repeatedly spelt tubullin (p259); reference lists at the end of each chapter, some in alphabetical order, others not, rather than altogether at the end.

Do advanced students need a textbook? Many courses prefer directed reading in the original literature and reviews, since ageing is rapid in the subjects covered here. Although the cover date is 1976, the book's literature survey just scrapes into 1974, comparing unfavourably with its most direct competitor, Lash and Whittaker's *Concepts of Development* (Sinauer, Stamford, 1974), almost as up-to-date, despite its earlier publication. The incompleteness makes the book less than ideal for either botanical or zoological courses) still in the majority?). Students may simply regard the 'irrelevant' chapters as money down the drain.

Despite these general doubts, and a few grumbles about details, I'd still heartily recommend this book for the excellence of many of its chapters, for its thoughtful overall philosophy, and for those sections which include examples from both kingdoms—they'll make biologists of us yet!

I cannot so easily recommend John McKenzie's *Introduction to Developmental Biology* (Blackwell Scientific: Oxford and London, £4.25; Halsted/Wiley: New York). This takes the view that students need to be cajoled into an interest in development by examining human development first. This may be true of medical students, but not, in my experience, of biologists. For an introduction, the book has too many unexplained anatomical terms (no glossary) and minor errors.

J. R. Downie

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Recipe books for crop production

Crop Production: Principles and Practices by S R Chapman and L. P. Carter (Freeman San Francisco, $15) is one of several similar books that outline the production of crops in the United States. All of them profess to introduce the principles of crop production before detailing the growing procedure for the major crops. For example, in this book by Chapman and Carter "Our philosophy has been that modern highly productive farming practices depend on an understanding of the principles of plant growth. When the principles are grasped, the practices follow logically." This is a commendable philosophy for the teaching of undergraduates in agriculture, but when a closer look is taken at these introduction principles (approximately half of the book) we find, in spite of a beautifully illustrated text, a rather thin veneer of elementary botany covering once again mitosis and meiosis, simple plant morphology, properties of soils, and so on which seem too common an occurrence in so many agricultural textbooks that try to cover too much material. To give two examples, where are the principles relating economic crop yield to plant density, which can explain seeding rate and crop establishment practices? and where are the principles of fertiliser response curves on which all fertiliser recommendations need to be based? As our present knowledge of field crop physiology gives a clearer understanding of many common crop practices throughout the world, it should now form the basis of crop production textbooks.

The second half of the book deals with production practices for each crop in the form of a growers' manual. This may be a useful source of reference, but one hopes that present undergraduates are not expected to learn and inwardly digest the factual details of crop-growing procedures. A further indication of thin coverage, is the allocation of only six pages of text to the maize crop, particularly since the crop originates from a country that produces 40% of the world's production.

In contrast, the latest edition of *Principles of Field Crop Production* (Collier-Macmillan, London, £7) by Martin, Leonard and Stamp gives some five times this coverage and would provide a better reference source. But in the final analysis, the recipe book approach to crop production makes this type of textbook no more effective than a farmer's manual for the country of origin. How, for example, does a University level agronomist, who has been reared on such a diet, adapt to unfamiliar environments, such as the third world, without an understanding of the principles of field crop growth?

G. M. Milbourn

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Microbial physiology . . .

Microbial Physiology (Basic Microbiology, Vol 4.) By I. W Dawes and I W Sutherland, Pp v+183 (Blackwell Scientific. Oxford and London, 1976) £4.25.

MICROORGANISMS can do so many things and can do some of them in so many different ways, consequently it is hard to give a short introductory account of microbial physiology which retains clarity and precision while covering a representative, if not comprehensive, range of material. The choice of topics in this book is judicious, but the coverage of them is somewhat uneven

The first chapter is a concise account of the structure and organisation of both eukaryotic and prokaryotic microbial cells with a useful digression on flagellar function This is followed by a chapter on the growth of cells and populations. Although the cell cycle and its relation to both production of new cell surface and DNA replication are well described, the part on growth of batch and chemostat cultures is rather slight A discussion of cell death and the mode of action of some antimicrobial agents is, however, included

The third chapter, "Substrate entry into the cell", prefaces four chapters on microbial biochemistry Energy production in heterotrophic organisms is covered in reasonable detail, and this is followed by a description of photosynthesis which is the worst part of the book, as it is not a particularly clear account, including an odd choice of detailed information and some unfortunate errors Equally, the chapter on monomer biosynthesis is patchy Some anaplerotic sequences are described in good detail, but it is not possible to deduce, for instance, how an organism might grow with succinate as sole carbon source The choice of biosynthetic pathways and their description on the other hand makes pleasant and informative reading

The next two chapters on macromolecular synthesis and the control of metabolism are also well done in general The concluding chapter is concerned with morphogenesis, showing the importance of this sort of phenomenon for microbiologists and what marvellous experimental systems microorganisms can provide for the study of a variety of morphogenetic problems

The standard of production of this book is fair the print is rather small but the text is divided by frequent subheadings There are plenty of illustrations, most of which are informative, although they vary in style and precision Thus, the effect of temperature on the growth rate of *Escherichia coli* is shown as an Arrhenius plot with fully described scales and with data points (which is good), whereas the induction of β-galactosidase by isopropylthiogalactoside is shown as a sketch graph without scales or data points, and is actually incorrect (which is bad)

The further reading suggested also leaves something to be desired Two to five review or textbook references are given for each chapter, but these do not always cover enough material For chapter two, there are no references on population dynamics and asynchronous culture techniques, although three references to cell cycle literature are given

This book may be of use for microbiologists in the early stages of an undergraduate course and as a concise survey for students of biochemistry and genetics. I think it is poor value for money at its present price.

C. F. Thurston

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. . . genetics . . .

Two recently published books on microbial genetics, aimed at first- and second-year undergraduates, cover essentially similar material, which can be broadly described as the organisation, function and recombination of bacterial and viral chromosomes and the molecular basis of gene action The emphasis of the two books is, however, very different. About 30% of D M Carlberg's *Essentials of Bacterial and Viral Genetics* (Charles C Thomas Springfield, Illinois, $24.75) is devoted to an interesting account of mutation and a further 30% to recombination systems in bacteria and viruses. About 40% of the new edition of J R S Fincham's *Microbial and Molecular Genetics* (Hodder and Stoughton: London, hardback £3.95, paperback £2.25, Crane, Russak New York), on the other hand, provides useful summaries of gene action, regulation, plasmids and DNA technology Fincham follows the now traditional sequence of the properties of DNA, chromosome mapping and mutation followed by the topics already mentioned, whereas Carlberg covers protein synthesis, regulation and mutation before basic features of genetic systems which inevitably results in a degree of cross referencing

As an introductory text Fincham's book suffers from not being self-contained Some of the basic material on chromosome mapping and bacteriophage biology is not clearly explained. For example, it is not very helpful to provide ascus analysis data from a four-point cross in *Neurospora* together with a bald statement in the text that "Table 2 illustrates the principle" (p31) It is also difficult to understand why there are no diagrams of the lytic and lysogenic cycles of bacteriophages when there are diagrams of life cycles of *Neurospora* and yeast, organisms covered in only a minor part of the book Another topic which lacks diagrams is the mechanism of sex factor insertion and chromosome transfer in bacteria In the preface the author hopes that the book will not be beyond the "ambitious beginner" in biology but one might add to that the "intelligent beginner", as I do not feel that this is the book to guide the average student through these aspects of genetics Although the author provides selected textbooks and original articles for advanced students he does not really cater for elementary students working at this level Reference to articles in *Scientific American*, *Science Progress*, *Science*, *New Scientist* and the *British Medical Bulletin* would have been more appropriate

Carlberg deals with his subject matter rather broadly and includes some interesting material on industrial and medical applications of microbial genetics His style is essentially that of a lecturer giving an outline of the material but "leaving much fine detail for the interested student to seek out in the literature" This approach has much to recommend it as the book is readable, interesting and clear, but it has the disadvantage that the book cannot be used to provide the detail usually expected in a course text

Carlsberg's book is more lavishly produced, which is reflected in the price. The diagrams, many of them original, are on the whole clear but some are below the standard expected For example, the drawing of an icosahedral virus on p286 is inadequate, the models for recombination are not good, and a diagram on p193 shows a diploid cell with a haploid chromososome number It is also surprising to find no mention of bidirectional replication of DNA The inclusion of a patent application is interesting but it is difficult to see the justification for the inclusion of ten pages rather than an abstract of the important points.

Neither book attempts to be fully up-to-date in controversial areas, such as molecular mechanisms for recombination, so that the material included should not date too rapidly It must be recognised, however, that most genetics texts only have a useful life of a few years Neither book can be strongly recommended for use as course texts although Carlberg's might be suitable for particular types of courses Its price, however, would prevent its purchase as a standard text For students studying genetics as well as microbial genetics, a more sensible purchase would be the second edition of M W Strickberger's *Genetics* (Collier-Macmillan : London, £6.30+0.95 pence for answer manual).

Bacterial, Phage and Molecular Genetics. (Springer Berlin and New York, DM23; $9 50), a practical manual by U. Winkler, W Ruger and W Wackernagel, translated from the German, is a very useful collection of experiments which complement well the manuals already available There are a range of experiments on the purification of bacteriophages as well as detailed schedules on density gradient centrifugation techniques Molecular aspects include the isolation and analysis of DNA from bacteriophages as well as experiments on mRNA. The use of ultraviolet light, one of the safer mutagens, is fully exploited to show lethal effects, mutagenesis and the induction of lysogenic and colicinogenic bacteria. Standard bacterial and bacteriophage genetics experiments are included as well as transfection of *Escherichia coli* sphaeroplasts with λ DNA. The final section deals with gene action and phenotypic expression with systems ranging from morphopoesis in T4 bacteriophage to regulation of β-galactosidase In addition to schedules for 25 experimental areas there are also brief introductions to background theory, useful references, five sets of problems and answers and (particularly valuable) 48 pages of the results expected from the experiments described.

This manual should prove very useful to lecturers attempting to broaden the range of experiments in microbial genetics courses. The book is clearly produced and usefully illustrated with graphs and specimen data sheets The experiments described are not as ambitious as some of the recent manuals on molecular genetics but this has the advantage that most of the experiments should be feasible in a department with average equipment.

B. W. Bainbridge

B. W Bainbridge is Senior Lecturer in Microbial Genetics at Queen Elizabeth College, University of London, UK.

. . . and ecology

Interfaces in Microbial Ecology. By K C Marshall. Pp. x+156 (Harvard University . Cambridge, Massachusetts, and London, 1976) £9.40.

PROFESSOR MARSHALL has written a book that is attractive to read, and which is concise, selective, and clearly written Microorganisms living at interfaces do so in a peculiar habitat; they are faced by unusual physicochemical stresses and must live a very different life from that of the bulk phase organism What then do we hope to learn from a book that introduces us to the microbial ecology of this habitat?

The history of physical chemistry abounds with studies on the properties of interfaces, there are also clear ecological distinctions between gas–liquid, gas–solid, and liquid–solid boundaries; living bacteria exist in all environments and so must encounter such interfaces regularly. Topics of this sort are fundamental enough to need careful definition and discussion in an introductory text. Professor Marshall does precisely this, guiding us adeptly through some fairly advanced physical chemistry in the first few chapters, being careful to define his symbols as he does so.

Later chapters are concerned with "non-specific interfacial interactions in aquatic and terrestrial environments" and "specific interfacial interactions in microbial ecology". Although some workers may not be convinced of the need for this distinction, Professor Marshall's treatment of aquatic and terrestrial environments is stimulating and provokes one to further research He discusses the nature of bacterial adhesion, the problem of nutrients at surfaces, and the habitat of sedimentary microorganisms, and considers such terrestrial topics as moisture content, the rhizosphere and mineral dissolution In his final chapter Marshall comments on the characteristics of oral and intestinal microorganisms, and lists some other microbial curiosities all of which prove entertaining.

Technically, the book is well produced, and the typographical layout is clear Almost all of the figures and tables serve to amplify the text, except perhaps Fig 1.1 and 5.6, both of which suffer from being reproduced on matt paper. The bibliography is representative and the only fault I could find in the text was unfortunately a reference to my own work with Professor Crisp, which I thought was slightly irrelevant in a paragraph on primary microbial films (p61).

In general, I found Professor Marshall's book easy to read systematically, as well as being enjoyable to dip into, and can recommend it to advanced undergraduates and postgraduate research workers

P. S. Meadows

P S Meadows is Senior Lecturer in the Department of Zoology at the University of Glasgow, UK.

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Pocket guide to practical genetics

Methodology in Medical Genetics: An Introduction to Statistical Methods. By Alan E. H. Emery. Pp 157. (Churchill Livingstone: Edinburgh, London and New York, 1976.) £6.50.

THIS is a compact little book (I have reviewed it bit-by-bit, carrying it around in my pocket), clearly printed and well produced. The style is painstakingly explicit, missionary in tone, and unpretentious in its objectives and exposition.

The scope might fairly, and with no slight, be described as a compilation of those methods which Professor Emery, a practical human geneticist of extensive experience, has himself used. They comprise: standard segregation analysis (including some of the newer methods not covered in the classical texts); linkage analysis with and without the Bayesian modification; principles, but not details, of genetic counselling; twin studies, quantitative methods; and some of the elements of population genetics. There are many tables of wide utility, at present eclipsed by the computer, but (if our grants remain unfunded) likely to undergo a revival.

The author's unwavering practicality spares us the pages of pettifogging middle-grade mathematics which to the eyes of mathematicians and non-mathematicians alike, blight so many books on analytical genetics. His practicality is also a weakness. A really good mathematician with a true desire to communicate to the non-expert would have produced a somewhat more illuminating book. Here there are no underlying statistical principles stated: methods are produced with no visible means of support. Maximum likelihood estimation (MLE) is used, but we are told nothing of its properties, a neglect which has its counterpart in some of the methods chosen. For instance, there seems scarcely any point now in the Lenz–Hogben method which has never been well defended (for example, as to the asymptotic normality of the test statistic), is not based on a sufficient statistic, and falls far short of maximal power. On the other hand the MLE method recommended for the frequency of a rare recessive gene is so badly biased as to merit a jack-knife correction; in small samples it far from follows a normal distribution, a property implicitly invoked on page 5. (A Poisson approximation with confidence limits based on the χ^2 distribution would have been preferable.) Again MLE of θ, the recombinant coefficient, is so biased for large θ as to make estimation of loose linkage hazardous in anything but immense samples: thus it scarcely matters what, if any, mapping function is used.

These defects are likely to leave the student, nurtured on this book alone, with little statistical sense and without either inventiveness or resourcefulness. This trend shows up in the method recommended for estimating the frequency of an X-linked recessive gene, which discards all information on female subjects. (Numerical MLE is straightforward, if a trifle inelegant.) The ABO problem is dealt with by Bernstein's method, which is absolutely efficient asymptotically but which would not be so for four alleles or more.

I am afraid, however, that few biologists are mathematically adventurous. No doubt Professor Emery knows his audience at least as well as I do and caters for them appropriately. With a good, well-informed, teacher as a safeguard, this book might be successfully used as an elementary text, and many more sophisticated geneticists would be happy to have it in their pockets for ready reference.

Edmond A. Murphy

Edmond A. Murphy is Professor of Medicine at Johns Hopkins University, and Director of the Division of Medical Genetics at the Johns Hopkins University School of Medicine, Baltimore, Maryland.

Integrated text on population biology

Population Biology. By Thomas C. Emmel. Pp xii+371. (Harper and Row: New York and London, 1976.) £11.95, $15.95.

As the author remarks in his preface, "part of the problem of writing a book on population biology involves the host of inter-relationships among the basic contributing fields of ecology, genetics, evolution and behaviour". Dr Emmel copes well with this problem, providing a clearly arranged up-to-date account for the "well-prepared, advanced undergraduate". The book would also serve the professional biologist needing to catch up with some aspects of this recently integrated subject.

The chapters deal with cycles of matter and energy, population genetics and evolution, population dynamics, dispersion, dispersal, age and sex structure of populations, life-history patterns, mating systems, behaviour, climatic effects, and interactions of populations in communities. The reference list of 1,400 or so items is strong on recent contributions, and the index is reasonably comprehensive. The illustrations are on the whole excellent, although a few of the line drawings are disproportionately tall, and several of the half-tone pictures fall short of their purpose—the zebras in 11-14 do not appear cryptic, whereas the cicadas in 8-2b do (but should not).

In a fairly advanced book presenting many modern ideas and examples, workers in this field will inevitably find things to disagree with, and also errors. We are told on p222 that populations emphasising *r* selection typically inhabit regions with variable climates, such as the temperate zones, and on p224 that most terrestrial vertebrates and perennial plants seem to be *K* selected, whereas most annual plants and insects are apparently *r* selected. The joint consequences of these two generalisations might well startle a biogeographer. Only later are we warned of the uncertainties of *r* and *K* selection theory.

Most of the errors I noticed were minor misprints, an omission of a text citation from the reference list, and so on. I regard the original meaning of "decimate" and the proper spelling of "repellants" (noun) as lost causes, but hope we may save *Rhagoletis* from being confused (p60) with the "frit" flies. More serious is the confusing blunder in Fig 5-16, in which the prey curve is labelled "predator", and *vice versa*. On p229, the net reproduction rate R_0 is said to be the number of surviving offspring per female; it should be female offspring. On p53, one of the equations for intensity of natural selection is badly misprinted.

Although the material, like the subject itself, is predominantly American, examples are taken from many parts of the World, including Britain. Some of the examples cited are difficult to present briefly, and in places (for example, pp137–8) the exposition is not clear enough.

To a British reader it seems odd to find no mention of life-table analysis and *k* factor analysis for diagnosing 'key factors' and density relationships among the successive mortalities in the life-cycle. If this (as it seems to me) is a serious omission, it is an exceptional feature in this generally comprehensive and well-balanced account. With its stimulatingly up-to-date treatment, I should think it worthy of a place in the short-list of books for students of population biology.

M. E. Solomon

M. E. Solomon is Head of the Zoology Section at Long Ashton Research Station, University of Bristol, UK.

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Feel for biological rhythms

An Introduction to Biological Rhythms. By J D Palmer With contributions by F A Brown, Jr and L N Edmunds, Jr Pp. xvi+373 (Academic. New York and London, August 1976.) $19 50. £11 90.

THE author describes this as an introductory book, pitched at a level between popular books and technical treatises, these two stools are difficult to bridge The enquiring student, to whom it is in part addressed, might find it difficult to evaluate for himself the evidence for many of the statements made. each chapter ends with some recommended further reading, but the text does not direct the reader specifically to the appropriate sources for the different assertions. This would indeed hardly be possible in a book with so wide a compass in such modest size.

Apart from general descriptions of the nature and properties of biological rhythms, circadian, lunar and annual, the author describes in more detail rhythms in unicellular and multicellular plants and animals, and the use of circadian clocks in animal, particularly in bird navigation, as well as some of the implications for man The popular appeal is contributed in part by anecdotes of the background of some experimental investigations, but also by such journalistic epithets as "flamboyant" experiments and the "callous" decapitation of cockroaches There is a similarly journalistic passage about the motivation behind human subjects for experiments, which in no way enhances the scientific value.

Little over half the book is written, however, by the author himself. It ends with two extended chapters, by F A Brown and by L N Edmunds, discussing the two rival theories that biological rhythms depend essentially on subtle geophysical rhythms, or that they are due to an internal timing mechanism. Dr Brown's constant advocacy of the former hypothesis is familiar enough, but his chapter is the least readable in the book A little of it is simply polemical, but the greater part consists of closely reasoned arguments demanding from the reader a highly critical attention More than one interpretation can be put on many of the experiments described, but it is convincingly shown that a wide range of organisms can respond to a variety of quite small geophysical influences whose detection was hitherto totally unfamiliar to animal physiologists

Dr Edmunds in the concluding chapter avoids a polemical adherence to the endogenous theory but considers various possibilities of its mode of operation. Simple and convincing evidence is given that, at least in some insects, the rhythmicity is controlled by a part of the central nervous system operating by the release of a chemical substance, and, that in mammals and birds the suprachiasmatic nucleus, operating by way of the pineal, plays a dominant or at least an important role. With regard to the fundamental question of the underlying circadian escapement, which has been shown to be present even in single enucleated cells, the three major theories are outlined. theories depending on a biochemical feedback loop, on transcription along a DNA template, and on ions and ion transport in membranes With the advantage of a limited field to cover, this chapter is perhaps the most successful in introducing biologists from outside this field to the major lines of thought, although in other respects, by virtue of the very broad coverage of different periodicities in such a wide range of organisms, the earlier chapters should be very helpful for those who want, as the author puts it, to get a "feel" for the subject **J. N. Mills**

J N Mills is Brackenbury Professor of Physiology at the University of Manchester, UK

Animal response to environmental parameters

Comparative Physiology of Animals: An Environmental Approach By Richard W Hill. Pp xii+656. (Harper and Row New York and London, 1976.) £9 90.

AFTER years with no suitable text for an undergraduate course in animal physiology, we are now confronted with a succession of such texts and the problem of choice This latest offering, by Richard W. Hill, is a very readable book intended for students beginning their study of the subject. Hill places his emphasis on the animal in its natural environment and on the physiological responses to the various environmental parameters rather than on the fundamental mechanisms underlying these responses The physiological principles and relationships are presented accurately but primarily in nonquantitative and descriptive terms, and the book should appeal to readers who favour this approach

The scope is ambitious since it embraces all animal groups aside from protozoans and metazoan parasites The chapter by chapter organisation is based on physiological functions or environmental factors. The opening two chapters on organisms in the environment and on energy metabolism set the stage nicely for the following chapters on thermal relations, salt and water exchange, excretion, gas exchange and circulation The book concludes with a series of integrative chapters on oxygen deficiency, high altitude, diving, exercise and rhythmicity These final chapters are particularly successful in tying together the earlier material while reinforcing the environmental theme of the book The coverage of the various topics is comprehensive although occasionally somewhat repetitive (for example, the section of salt and water exchange). But students will appreciate the careful exposition of the concepts and the extensive use of appropriate experimental data illustrating the concepts The more inquisitive students, however, may be frustrated by the fact that practically none of these experiments are referenced and most are not identified with the names of the investigators involved. Potential subscribers are therefore forewarned to put their own knowledge of the literature in order before assigning this text to their students. Some readers may also be troubled by the absence of any detailed discussion of the neural and endocrine systems, and the consequent patchy and superficial treatment of the role these systems play in the physiological response to the environment In spite of these omissions, I found the overall organisation and content of the book to be quite coherent. It is an excellent text particularly suited for courses attempting to bridge the gap between physiology and ecology

This book is not without value for more advanced readers as well Hill's emphasis on the animal's response to its natural situation should remind those of us who study 'non-laboratory' animals in the laboratory to design our experiments carefully with the objective being to learn how these animals function in their natural milieu Conversely, we must learn all we can of our particular animal's normal milieu in order to design useful experiments These are also important lessons for students just beginning their studies in the field, and this book should serve that purpose well, while at the same time providing a sound introduction to animal physiology **Donald C. Jackson**

Donald Jackson is Associate Professor of Medical Science at Brown University Providence, Rhode Island.

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Human information processing

Memory and Attention: An Introduction to Human Information Processing. (Series in Psychology.) Second edition. By D. A. Norman. Pp. xiii+262 (Wiley: London and New York, 1976.) Clothbound £8.45, $15.45; paperback £3.95; $7.20.

Donald Norman's set of seminars on *Memory and Attention* have been useful to university teachers since they were first published in 1969. It is a pleasure to have a second edition, so adapted and extended that it is now virtually a new book. It is arguably also a different kind of book, since it is now probably the best and most cheerful introductory text one could recommend to a layman who wishes to understand how work on artificial intelligence and information processing is currently applied to psychological problems.

Among a large, mostly very recent, literature this book has distinctive virtues. First it is by a single author, who writes well. Second, it is short. Most importantly it is a good natured book, which seeks to be eclectic in appreciating the values of a wide range of different approaches. It retains enthusiasm without losing balance. It is a book to teach *with*, although possibly not a book to teach *from*.

Norman hopes that "the study of human information processing is becoming the study of the human" but aptly warns that "one must always be wary of comparisons of human psychology with any man-made device, most especially with computers. The human mind is not a computer, and the differences between the two far outweigh the similarities". Norman is also rare among workers in Artificial Intelligence in having a scholarly interest in the history of psychological problems. To quote one of his favourite authors, William James, he also has an appetite for the "more nutritious objects of attention" in psychology.

He is willing to accept the kinds of questions which students bring to psychology from their discursive reading, and while pointing out that some of them are unprofitable, is willing to discuss them seriously and in an illuminating way. Thus we find comment (and very useful references) on the topics of mystical experiences (Carlos Castenada *et al*), hemisphere differences (Ornstein *et al.*), mnemonic systems (an entire very useful chapter and excellent bibliography), pathologies of attention and of cognitive processes, and even the art of juggling. To some extent these are embroidery on a serious text, and they do not occupy much space. But they are not mere decoration. They provoke useful commentary, and show a recognition that the enthusiasms which bring students to psychology need not be stifled, but can mature into more usefully directed intellectual curiosity.

It is easy to dismiss such topics as irrelevant to current empirical work, and to depress students by insisting that psychology has nothing to do with the study of human experience and is concerned instead with the description of "performance" or "behaviour". Norman's caveat for psychologists should be taken seriously by all students, and properly understood is an antidote to the depression felt by research workers who may feel that they are wasting time on the precise definition of trivial problems. He points out that human beings have been the object of their own attention for a very long time, and that perhaps "We cannot discover anything surprising . . . It would be peculiar if our careful study of thought processes led to results which are at odds with our intuition". But he further points out that the bridging of a gap between a nebulous appreciation of generalities and the precision of predictive mathematical theory is an exciting, and ultimately a satisfying, occupation for a scientist.

The work follows the format of the first edition in presenting related readings with commentary and further development. The chapter on attention is now extended by a discussion of recent work by Kahneman and Moray, with useful remarks on the problems in terms of Bobrow and Norman's own distinction between "data-limited" and "resource-limited" processing. The previous section on short-term memory has been extended to cover the Craik–Lockhead idea of levels of processing. There are excellent chapters on pattern recognition programs sensibly restricted to detailed analysis of the problems encountered with a *single* new system (Reddy and Newell's Speech Understanding system). A section on semantic networks forms part of an excellent chapter on representational systems. There is a good introduction to imagery, in the same vein.

The book is research oriented rather than didactic, and ends as it should with a set of problems outstanding (Unfinished Business). Not the least of its values to students and to lecturers is the care with which really comprehensive bibliographies are assembled and annotated.

Patrick Rabbit

Patrick Rabbit is Lecturer in Psychology at the University of Oxford, UK.

Memories are made of this

The Psychology of Memory. (Basic Topics In Cognition Series) By Alan Baddeley Pp. xvii+430. (Harper and Row: London and New York, 1976.) Hardback £10.45; paperback £5.45.

AT LAST, a textbook on memory which one can recommend on its own, without three or four other volumes. The topic is a big one, ranging from the strange phenomena of clinical repression or 'photographic memory' at one end, to the effects of disrupting protein synthesis at the other. In the middle, there are groups of experimenters measuring the accuracy of memory in prescribed conditions, who are steadily building up the rules by which the system functions, whatever its physiological base. Even these groups are subdivided, some looking at associations between single items; some at memory for sentences and propositions, some at the similarities and differences of long-term and short-term retention; some at the importance of the particular form in which an event is encoded (roughly, perceived) at the time it occurred; and so on.

The difficulty is that each of these areas is still relatively unconnected with the others. All of them are advancing rapidly, it is hard enough to form a clear picture of the sense emerging from the mass of experiments in one field, without venturing into the others Most books therefore concentrate on one sector, often doing it well Yet the problem for the student or non-psychologist is that the picture he gets from such a book is one-sided. Even the slighter and more introductory texts often confine themselves to one approach, but merely replace detailed evidence by bald assertion. It is not surprising that even professional psychologists are sometimes unable to see the wood of progress for the trees of particular experiments.

Dr Baddeley's book, however, is different. It is not merely that his index mentions Freud as well as Ebbinghaus, Bartlett as well as Chomsky, acetylcholine as well as mnemonics. He could scarcely cover them all exhaustively; but the novel feature is that each field is mentioned appreciatively His own most familiar area is perhaps that of long-term as against short-term memory, but he is sympathetic to all, and though the amount of space he gives to each may get less as it becomes more remote from his own specialised expertise, the reader is left aware of every approach. Furthermore, the writing is beautifully lucid, and yet each point is based always on a well-selected experiment rather than generalities He is also accurate, which cannot be taken for granted.

Such a book should appeal to anybody who is prepared to read nearly four hundred pages on memory; despite the clear and attractive style, it is all information rather than exhortation. Readers will therefore need to come to the book already convinced the subject is important. If, for example, they are doing an honours degree in psychology, or worried by the memory failures they meet in neurological practice, they cannot do better than pick on this book as their main source of information.

As favourable a review as this one is, some criticisms are still necessary as a guarantee of a proper standard. First, the sequence of topics is rather arbitrary, and one feels the chapters could be read in almost any order. This seems to stem from boredom with the conventional approach of starting with the arrival of a stimulus at the senses, and following it through. Perhaps this convention has some merit in the absence of any other organising principle

Next, the distinction of short-term and long-term memory is surprisingly cavalier, and relies heavily on the very rapid forgetting of recent as opposed to more remote events But this phenomenon, as has often been said, does not justify such a distinction. Dr Baddeley is now used to distinguishing far more than two kinds of memory, but some students will still be struggling with their tendency to parsimony, and could do with a clearer presentation of the reasons for abandoning a single homogeneous memory store

Finally, the excellence of the book for teaching means that Dr Baddeley's own ideas have not been let loose. We could do with expansion of his brief suggestions about a modified interference theory, or about the role of talking to oneself for working memory during mental arithmetic. Perhaps a specialist monograph might follow? Yet this survey is itself a contribution to the subject since it reminds us all that research in this field has come a long way in twenty years.

Donald E. Broadbent

Donald Broadbent was Director of MRC Applied Psychology Unit from 1958–74, and is now on the MRC External Staff at the Department of Experimental Psychology, University of Oxford, UK.

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Substance of demography

A GROWING interest in our species is reflected in the recent appearance of several general textbooks on demography. *Population: Analysis and Models* by L. Henry (Arnold: London, hardback £9.95; paperback £4.95) adds a valuable member to the company and demonstrates the continuing strength and vitality of the French contribution to these studies. Although in agreement with the translators that no demographer can be without a reading knowledge of French, they are to be congratulated on providing an excellent translation for those whose command of the language is imperfect. This is nevertheless an imposingly technical text, with considerable mathematics, although for the less numerate progress is eased by the usually clear and adequately explained steps. The text introduces a useful and interesting graphical tool, the Lexis diagram; elsewhere, however, more might have been explained by graphical presentation: the treatment, for example, of age–sex pyramids seems to be unnecessarily brief.

M. Henry's approach follows in general the now common 'longitudinal' study (developed by American and British demographers), the examination of data based on generations rather than the more limiting and older 'cross-sectional' method using only data from periods of a few years. The longitudinal approach is useful in enabling the link between population structure and movement to be clearly developed, and is well suited to the sociological emphasis of demography in the English-speaking world.

The description of the book on the jacket stresses that it covers the conventional substance of demography—source materials, methods of description, nuptiality, fertility, family formation and mortality (note the order!)—but also emphasises the author's attention to migration and its effective integration with population structure. Amid all these goodies, however, one dish seems inadequately prepared. There seems to be a general weakness among demographers that they give little depth to the spatial elements of population—on p 13, under "Analysis of the Results", two meagre paragraphs on geographical distribution are so trite that they would have been better omitted. But even later in the text, especially when dealing with migration, the spatial element of which is so pronounced, there are statements over which geographers would readily take issue with the author. Regional variation in dynamic patterns seems to go inadequately recognised.

Part II, the final but lesser half of the book, is an introduction to demographic models and, perhaps importantly, a statement as to why models are of value and why they should be considered by demographers. Much of this section is centred round Lotka's theory.

The French version of the UN multilingual demographic dictionary adds a final phrase to the definition of demography that it is a study "from the quantitative point of view"—M. Henry has made just this point.

Methods and Materials of Demography by Shryock *et al.* (Academic: New York and London, $16.50; £9.55) is a condensed version of a two-volume work of the same title originally published by the US Bureau of the Census in 1971 with subsequent later editions. It is now designed primarily as a textbook for courses on various aspects of demography, but it is equally useful to professional workers with occasion to understand or use demographic data for analytic studies

The author of the abridgement claims to have presented the methodological material in the simplest mathematical form in order to make the text as useful as possible, a claim which even cursory examination of the text supports. For the most part a modest understanding of algebra and statistics will suffice to carry the user through the relevant sections.

Although originally a publication of the US Bureau of the Census, the illustrations and coverage are not solely restricted to American examples, but considerable weight is given to the problems of demographic assessment in the less developed countries. The author does, however, stress that the methods and measurements worked out for the USA are to a considerable degree "culture free" and may serve as well for any other country. In this condensation a necessary choice of inclusions and omissions had to be made, but a balance between formal demography and social and economic demography was sought. In this search, the author has been reasonably successful.

In general the step-by-step illustrations of examples are based on actual figures, although necessarily hypothetical statistics have had to be used in some instances. Where official statistics have been used they have mainly been taken in their form as published, apart from a few instances of simplification. In this condensation, less emphasis has been put on historical development, the recommendations of international organisations and the practices of national statistical agencies, whereas the more technical and mathematical aspects of demographic analysis have been reduced in emphasis.

The book is, however, not without its weaknesses. Certainly chapter 5—Population distribution Geographic areas—leaves many gaps, especially in the discussion of the important aspect of mapping distribution. The range of techniques examined is quite inadequate, and unfortunately some of the less impressive and informative methods are chosen for black-and-white line diagrams.

Nevertheless this is a fact-packed text invaluable to the do-it-yourself census maker, and brings together much material otherwise diffusely scattered. For any serious course on the principles of demography it should prove a useful if expensive textbook.

R. E. H. Mellor

R. E. H. Mellor is Professor of Geography at the University of Aberdeen, UK.

- *Growing Points in Ethology* (Cambridge University: Cambridge), by P. P. G. Bateson and R. A. Hinde, has also been published in paperback, price £5.50. (For review, see *Nature* **265**, 385, 1977.)
- In the review of *Environmental Chemistry* (Academic: New York and London; *Nature* **264**, 818, 1976), the price was incorrectly quoted This should read $15.95; £11.30.

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Comprehensive geomorphology

Analysis of Landforms by C R. Twidale (Wiley Australasia: Sydney, New York, London and Toronto, £13.50; $27) is intended for use as a text at advanced school level and for those taking degree courses in geography, geology, Earth sciences and environmental sciences. It sets out to provide a general text with an emphasis on Australia but with an adequate coverage of world examples.

The method of presentation leans heavily on photographs and diagrams and the book is lavishly illustrated. Yet its size is such that the written discussion is ample, as well as being of high quality and backed by discreet quantification. To keep the volume within manageable length, the nature and methods of close analysis are illustrated from a relatively few selected landforms, such as flared slopes, but here again the spacious text allows the general field to be covered in considerable detail.

The voluce opens with a discussion of the scope, problems and methods of landform analysis and reveals a preference for multiple working hypotheses. The second part deals with structural geomorphology, including pseudo-structural forms; the third with geomorphological processes and climatic geomorphology; and the fourth with changes in time or historical geomorphology. The concluding part summarises the number and variability of factors involved in landform creation.

So it is better to travel hopefully than to arrive, but the student with this attractive companion will enjoy and benefit from the journey, especially as its ample text is strengthened by plenty of references and an enlightening geological appendix. The cost may seem high to those who have not seen the volume, but in fact its lavish visual aids and large-format pages make it good value The printing—carried out in Singapore—is handsome and effective and adverse criticism must be minimal. It is misleading, however, to use Mercator's cylindrical projection for global distributions such as plate tectonics. This graticule has no uses whatsoever except for finding compass directions between localities, and students will never truly understand global distributions and movements until they see them on equal area projections.

Another recent book, *Geomorphology from the Earth* (Harper and Row: New York and London, £14.20), is a compact synthesis of geomorphology and stems from Chicago, an early citadel of landform studies. Its author is well known for his illuminating writings on the physical environment and its significance for Earth sciences ranging in diversity from archaeology to biology. He assumes—quite rightly—that an understanding of the surface forms and textures of our planet is a necessary ingredient of a liberal education, that landforms and the processes that create them are fascinating to scientists and curious people everywhere; and that persons of all ages and educational backgrounds gain from geomorphology a new appreciation of other environmental sciences I have known students with souls so dead as to exclude empathy, males more curious about velocity than volcanicity, and females more interested in finery than feldspars, but have no intention of being an ancient mariner at a geomorphological feast for this is attractive fare laid out in an enticing way.

Professor Butzer keeps his aim of a general introductory text clearly in view and includes the essentials, critical interactions and brief overall perspectives New findings and modern developments are incorporated throughout with a judicious use of mathematical expressions and, for readers who want more specialised aspects, with ample international sources in lists of references The contents cover a broad field and, being confined to a moderate length, vary in intimacy of detail, the tendency being to elaborate most on forms and processes of greatest human significance. Commendably, space is found for a historical summary of modern concepts, before dealing with the lithosphere, weathering, soils and the sculpture of hillslopes There follow discussions on topics such as fluvial dynamics and erosion, the work of glaciers and of waves and the importance of crustal movements. Separate chapters are devoted to periglacial landforms and to the characteristic landscape assemblages of mid-latitudes, deserts and tropical regions.

The high quality of the explanations, diagrams and photographs and the emphasis on the essential rather than the exotic will be admired by geomorphologists. Perhaps general readers may not appreciate the wisdom in the compilation but they will realise that geomorphology is a fascinating science and of great human significance The text, in spite of its price, will also be valued as a stimulating revision for students already acquainted with the subject.

Robert P. Beckinsale

Robert Beckinsale is an Emeritus Leverhulme Fellow and a Special Supernumerary Fellow of University College, Oxford, UK.

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Perspectives on physical geology

PHYSICAL GEOLOGY, defined by S. Judson *et al.* in *Physical Geology* (Prentice-Hall. Englewood Cliffs, New Jersey, $14.50) as a discipline which "addresses itself to the composition, classification and origin of earth materials, and to the physical and chemical processes which go on at or beneath the earth's surface", covers a formidable range of material. At the small scale it deals with soil creep and at the other global tectonics. In addition it seems that under its umbrella one must attempt to cope with the study of resources, with environment, and with the characteristics of other planets.

It would be remarkable, given the scope that this offers, if three books on physical geology were to cover the same material in broadly the same way. Three recently published volumes however, are remarkable in just such a way. They have plainly been aimed at the same market (majors and non-majors in a one-semester or one-term course at the American college level), they have broadly similar format (multi-colour printing, many illustrations, good type); they all attempt to cover similar ground, and they all make commendable efforts to be comprehensible and attractive.

The differences between them are of a cosmetic rather than a fundamental nature. J H. Zumberge and C. A Nelson in *Elements of Physical Geology* (Wiley. New York and London, £7.20; $14.40) offer the best colour illustrations of rock types and minerals together with an illustration of a seemingly topless Hawaiian surfer J E Sanders *et al* in *Physical Geology* (Harpers College, Harper and Row London and New York, £10.45) offer flip drawings of Pangaea on the top corners of some 40 pages to "present the idea of drifting continents as vividly as possible", together with extracts from other writers (which they term "field trips"). And Judson *et al.* both pose and answer questions for the student, but (presumably why on a page for page basis they are cheapest) they offer slightly less glitter.

A second remarkable feature of such books would be for them to range successfully and adequately across the great range of material they seek to cover. Broadly speaking all of them do this, and broadly speaking their content and their guides to further reading are up-to-date They all seek to put across the rudiments of mineral characteristics, rock types, volcanoes, weathering, mass movement processes, rivers, orogeny and plate tectonics Judson *et al.* use the plate tectonics concept as a framework for much of their discussion whereas it is less prominent in Zumberge and Nelson and in Sanders *et al.*

Inevitably there are inadequacies, omissions or lack of awareness of recent work. Rates of geomorphic processes are consistently neglected; climatic changes are given scant attention; coral reefs are accorded little prominence; Davis's great cycle of erosion is relegated to the very briefest of mentions, and the nature of water movement in drainage basins (particularly throughflow and runoff generation) is hopelessly out-of-date in the way that it is treated.

Overall there is not a great deal to choose between these three texts. They all offer clear, well-produced introductions to their views of physical geology. Probably their main use in Britain will be as texts for sixth-formers, but most University undergraduates would find shorter texts covering specialised topics at a greater depth more useful.

Andrew Goudie

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Structural geology

An Outline of Structural Geology by B. R. Hobbs, W. D. Means and P. F. Williams (Wiley New York and London, $20; £10) is intended to replace Sherbon Hills' *Outlines* (Prentice-Hall, 1972) as a comprehensive text on structural geology for undergraduates. The involvement of three authors, all well known and experienced research workers, is perhaps a comment on the rapid growth in the complexity and diversity of the subject over recent years. Some years ago structural geologists were chided with scientific myopia, specifially, for having 'played' with minutiae while others discovered plate tectonics. Fair comment or not, this reminds us that when subjects develop toward a labyrinth of narrow specialisations there is an urgent need for a survey of the whole field of activity.

The plan of this book is not novel, indeed it has much the same lay-out as Sherbon Hills' and its success depends on the quality of treatment of familiar topics. It starts with mechanical aspects and stress–strain theory, and progresses from minor structures (microfabrics) through folds, faults, foliation and lineation to major structures and, finally, plate tectonics. The 'bridge' from small to large is in parts of a useful, but rather brief, chapter on "structural associations", that is thrust belts, block faulted regions, and so on. There is also an essential chapter on geometrical analysis and this topic, including stereographic analysis, is continued in an appendix.

It is a pleasure to record that the authors' surefooted approach, with its mingling of fact and comment, hardly ever lets them down. Fortunately, the authority which is evident in individual sections of the book is not spoilt by any overall lack of balance which is I suppose a danger when several authors are involved. Of course it is not perfect. For this reader, the splendid section on microfabrics is not always matched elsewhere in the book and it is surprising to find so little on fluid pressures, or on pressure solution, or on techniques for estimating strain, shortening and displacement in orogens. My impression is that the authors are happier on the minor structures than with major structural phenomena. Their chapter on plate tectonics, however, is quite admirable.

The illustrations are vital in a book on structural geology and here there are many excellent diagrams and photographs, and very few disappointments.

The authors have designed their book for undergraduates. There are several potential rivals even if we exclude J. G. Ramsay's classic advanced book (*Folding and Fracturing of Rocks*, McGraw-Hill, 1967) and that by F. J. Turner and L. E. Weiss (*Structural Analysis of Metamorphic Tectonites*, McGraw-Hill, 1963), which has never been suitable at this level. The most recent editions of the respected books by L. U. de Sitter (*Structural Geology*, McGraw-Hill, 1964) and M. P. Billings (*Structural Geology*, Prentice-Hall, 1972) cannot be considered as truly modern in approach.

The books by E. W. Spencer (*Introduction to the Structure of the Earth*, McGraw-Hill, 1969) and P. C. Badgley (*Structural and Tectonic Principles*, Harper and Row, 1965) share some of the virtues of de Sitter's book in that they include 'potted' accounts of classic regions for the study of deformed rocks and this must be an asset for the hard pressed undergraduate. But the present book must surely take precedence over these because it goes further toward the goal of presenting structural geology, not only in modern terms but also as a coherent subject based on physicochemical laws I think the research worker will also benefit from this reliable and well produced book **M. R. W. Johnson**

M. R W Johnson is Reader at the Grant Institute of Geology, University of Edinburgh, UK.

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10 March 1977

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Carter and the test of energy

For the first time since the Arab oil embargo, the United States Government seems ready to consider adopting a comprehensive energy policy, one which involves efforts to curb the nation's profligate consumption as well as attempts to step up domestic energy supplies. Such a policy is long overdue, but it will severely test the will and the political skills of the new Carter Administration to put it into practice.

Although both Mr Nixon and Mr Ford paid a good deal of lip service to the need for energy conservation during their terms in office, their energy policies consisted essentially of throwing large sums of money into the supply side of the energy equation. Indeed, when Mr Nixon launched his infamous Project Independence, he even insisted on making absurd claims that American technology would free the United States from relying on imported oil to meet its energy needs by 1980. The demand side of the equation was badly neglected, perhaps for understandable reasons.

The United States has grown up with cheap power, with the ethic that bigger means better, and with the philosophy that the government has little right to meddle in the workings of the so-called free market. All that would have to be changed if a serious attempt is made to curtail energy demand. Mr Nixon, who was already under siege when the oil embargo struck, probably lacked the political ability to pursue effective conservation policies—in any case, he didn't make much of an effort. And Mr Ford suffered the handicaps of being an unelected President who belonged to a different political party from the one which controlled the Congress and who had an abiding faith in the free market.

President Carter may be different. His campaign rhetoric was peppered with promises to develop an energy policy, and he has repeatedly warned that it will require considerable 'sacrifice' from the American people. He also comes to office riding a tide of goodwill and—perhaps most important—this year's extraordinary winter has provided a more vivid argument for conservation than a thousand Presidential statements. If Carter doesn't manage to put an energy policy into effect, it would be a serious indictment of his Administration's political ability.

So far, Carter's efforts are promising. The first major instalment of his energy policy came last week when he proposed legislation to consolidate most of the federal government's sprawling energy bureaucracies into one Cabinet-level department under the direction of James Schlesinger, former head of the Atomic Energy Commission, the Central Intelligence Agency and the Department of Defense. Though the proposed arrangement is eminently sensible, the bill would clump together agencies and programmes which are now handled by different Congressional committees. Some jurisdictional disputes may therefore delay passage of the Bill, but approval is nevertheless anticipated.

Once in place, the Department of Energy should be in a better position to plan both conservation and supply policies. That's where the second instalment of Carter's energy policy comes in. He has promised to unveil a comprehensive energy plan on 20 April, with particular emphasis on conservation. A preview of at least some of the elements of that policy was provided last month when Carter sent Congress a number of revisions to the budget proposals which Mr Ford left behind.

As far as research and development are concerned, Carter's policy will concentrate on efforts likely to produce results in the relatively near term. Thus, long term efforts such as the breeder reactor programme, nuclear fusion efforts, and the development of large scale solar power plants, would be cut back and the money reprogrammed into energy conservation and into efforts to get already-developed technologies, such as solar heaters, into the market-place. The justification for such moves is that it will probably be more difficult to commercialise new energy technologies than to develop them, and government incentives are required to feed the growth of, say, a solar energy industry. Those arguments are likely to find favour in Congress.

As for curtailing energy use, the Administration can pursue only a limited number of technological fixes, such as forcing the automobile industry to make more efficient cars and encouraging the use of battery-driven vehicles. But the most effective energy conservation measure would be to increase energy prices through the use of federal energy prices through the use of federal energy taxes.

Such a move would, however, be very difficult to get through the Congress and it would also have to be coupled with a system for returning the tax revenues back into the economy, preferably in a manner which would mitigate the burden on low income groups. It is estimated that every cent of tax on a gallon of gasoline would raise about $1,000 million in revenue; a large tax could have a depressing effect on the economy. One idea which is being floated is to return some of the tax revenues into energy-saving measures such as income tax credits for home insulation, construction of mass transit systems and so on. In any case, the Carter Administration has already begun to leak the fact that it is considering raising energy prices in order to prepare the American public for the inevitability of a long overdue move away from the present artificially low prices of energy. □

Between the devil and the deep blue sea

Robert W. Cahn, Dean of the School of Applied Sciences at the University of Sussex, offers this comment on the prospect for fusion power

In the anguished debates on the future, if any, of nuclear fission power, various recognisable strands emerge among the opposition. Some pin their faith on the improved technological exploitation of familiar fossil sources, principally coal; others would drive us headlong into the solar economy, often conceived in terms of 'small is beautiful' and a rural life of log-cabin simplicity; yet others accept the need for high technology on a very large scale if the sun is to yield domestic warmth, electricity and liquid fuel on an adequate scale.

All three groups, however, have one characteristic in common. When faced by hard statistical or technological criticisms, they declare that we need only keep going for a few decades and then the boundless energy "from seawater"—that is, nuclear fusion—will become a reality and all our worries will be over. Although they do clearly recognise that the method is not altogether simple, even the most resolute proclaimers of radioactive doom seem to have convinced themselves that fusion power will be gentle and on a homely scale, and that radioactive pollution will be a thing of the past when dirty fission gives way to clean fusion. Probably the fact that fusion takes place in the sun and that the sun is the emblem of cleanliness and of life itself, has something to do with this touching faith. We are all to some degree subject to the ad-man's technique of fallacious association.

There is no dearth of popularisations of this very difficult and complex topic, but they all concentrate on two aspects of fusion—how to confine the plasma, and how to heat it to ignition temperature. Even within these limits, they tend to concentrate on scientific principles and not on engineering realities, whereas the current critcism of nuclear fission concentrates on real and alleged engineering weaknesses. Nuclear fusion has progressed far enough now towards the design of a self-sustaining reactor to make it both possible and necessary to see the problems in realistic terms. The plasma confiners—which indeed have a ferociously difficult task—until recently have hogged all the attention, to the neglect of the problems of energy balance (achieving a positive net energy output), container strength and fatigue damage, lithium supply, reactor cooling, injection of gas into the plasma without quenching it, and especially the radiation damage inside the reactor itself.

That this imbalance is now being repaired is primarily due to the efforts of the Energy Research and Development Administration (ERDA), which has complete control of fusion research in the United States. ERDA has prepared a detailed R&D plan extending to 1990, and in outline to the end of the century. ERDA's achievement is to have set out this programme without committing itself to any particular design of reactor, and yet to have identified the central problems which will have to be faced irrespective of the design. To do this, ERDA's predecessor in 1967 invented the concept of the 'reference design'. This is an outline based on scientific principles, as presently understood, set out in such a way as to identify, as quantitatively as possible, the whole range of engineering problems that will need to be mastered. Such a reference design is particularly illuminating when applied to the doughnut-shaped Tokamak, which in purely scientific terms is today probably the most hopeful contender among the rival approaches.

On the basis of such a reference design, the problem of securing the deuterium (by electrolysis of water?) and tritium from a metre-thick breeding blanket of molten lithium surrounding the first wall of a doughnut-shaped plasma device, can be analysed, not least in terms of lithium availability; lithium is a scarce element. One day it will also be necessary to include in the energy balance equation not only the energy cost of extracting tritium from irradiated lithium but also the electricity costs of electrolysing water to extract deuterium, a calculation which never seems to be reported.

Probably the most alarming engineering problem is that of radiation damage. In the much-criticised fission reactor, neutrons, gamma rays and fission particles remain inside the reactor volume, and indeed the fission products remain inside the nuclear fuel itself. In a Tokamak, all the neutrons, moving with a kinetic energy of some 14 MeV, beat upon the first wall: the scale of this bombardment dwarfs anything in a fission reactor. The wall can be sputtered away, especially by the novel hazard of 'chunky sputtering' which involves the physical removal of thousands of atoms at a time.

A great deal of research is now in progress on this group of problems in the United States (some also in Britain), and considerable advances have been made in improving the radiation resistance of high-strength refractory alloys. But it is certainly an error to regard fusion reactors as generating little radioactivity: there will be a great deal of induced radioactivity, though not from fission products, and it will be just as crucial as in the case of a fission reactor to contain and seal off this radioactivity from the outer world. (It seems likely that this hazard will not be long-lived). Apart from this, there will be just the same hazards of pinholes developing in inaccessible locations as have been found with prototype fission-breeder reactors, leading to deterioration in cooling efficiency or vacuum quality.

Fusion may prove to be a practical source of energy—the new issue is now as much in the hands of the engineers and metallurgists as of the plasma physicists. If it comes, it will be an immeasurable boon but it will also bring with it problems of pollution, energy imbalance during construction, resource depletion, sabotage and potential stoppage such as we find with every other source of energy, including solar techniques such as the large-scale fermentation of timber to make liquid fuel.

For Blake, energy was a mark of the divine. Milton knew better: Satan has energy too. Whether the external flames are fed by coal, oil, uranium or deuterium is a question which must tax the physical theologians of the future. ☐

IPP

Fusion technology: installing coils on a torus in Germany

Europe's nuclear debate (1)

Austria: a case study

Helmut Hirsch and **Helga Nowotny** assess Austria's nuclear energy information campaign

NUCLEAR energy has become a topic of interest in Austria much later than in other countries, so her politicians and scientists have been able to study the development of the issue for some time. It has also come at a time of social stability, when even a small disturbance would not go unnoticed. This, and the manner in which the authorities control and institutionalise conflicts, help to make Austria an interesting case-study. For unlike the experience of other western countries, Austria's nationwide nuclear debate was initiated by the government. It organised the *Informationskampagne Kernenergie* (nuclear energy information campaign), and from being a topic important only to a few environmentalists and dwellers near proposed reactor sites, the subject became a national concern drawing more and more citizens into action groups.

The first steps were taken in July 1975. The Federal Chancellor's Office was responsible for the organisation, and a committee of civil servants, chaired by the head of the Energy Section of the Ministry for Trade, Commerce and Industry, Dr W. Frank, took over the scientific preparation. An exhaustive catalogue of questions was compiled, divided into well-defined subtopics embracing economy, safety, environmental and biomedical questions. In accordance with frequent practice and to avoid suspicions of manipulation, the cooperation of nuclear energy critics was sought, among them the Austrian Nobel-prize winner Professor Konrad Lorenz, and Doz. B. Lötsch, the head of the Viennese Ludwig Boltzmann-Institute for Environmental Sciences.

Each of the subtopics was to be dealt with by a group of experts, consisting of critics and promoters of nuclear energy in equal numbers (about three of each). This pro–con classification later proved to be an over-simplification: many scientists don't fit into either category, many more belong clearly to one side but refuse to admit it. Preliminary discussions would clarify as many points as possible, while the open problems would be left for the public debates. Finally, a report was to be produced containing relevant information, answers to questions on which consensus was reached and recommendations for further research. Although the organisers believed scientific controversies arose simply because there was not enough data (unless they concerned an issue that wasn't a real scientific problem), this later proved a rather naive conception as controversies also exist about which data can be regarded as relevant and about the appropriate framework for their interpretation.

Emphasis on independence

Strong emphasis was placed on the independence of the experts. People employed by electrical utilities, the nuclear industry, or concerned with the licensing of nuclear power plants participated only in internal debates as consultants. Civil servants were also excluded as experts from the various ministries because they would have to contribute in subsequent parliamentary debates. Thus, the government could remain non-committal, not being responsible for what independent experts choose to say or write. For the same reason, politicians from the governing Socialist Party participated little; their frequent absence from the debate was often criticised.

Promoters of nuclear energy felt that the campaign would emphasise the disagreement between experts and only increase public fear and suspicion. But most critics failed to appreciate the genuine attempt to avoid the errors made in other European countries. Hence the emphasis on sub-groups working on different topics—too often discussions had remained superficial and disorganised; and the attempt to balance the expert groups between pro and con—too often 'information' had proved to be only state-sponsored propaganda.

The government took the final decision to initiate the campaign on 17 February 1976. As there were ten subtopics, ten moderators for the expert-groups, mostly university professors, were selected by Dr Frank. As every expert has a personal opinion or a professional bias on the subject, it was impossible to choose strictly neutral moderators. Nuclear physicists and economists specialising in energy problems tended to be pro-nuclear, whereas the rest were critical or at least suspicious. Most felt ill at ease in their positions.

The internal debates, organised and conducted by the moderators, started in June 1976. No questions were excluded from the public debate because it was impossible to define clearly solved and unsolved problems.

To prepare the public, the Federal Press Service issued a booklet providing basic information, *Nuclear Energy—A Problem of Our Time.* Although controversial issues were reserved for the subsequent debates, disagreement among the authors (mainly the moderators) was considerable and many passages were tentative and noncommittal. Nevertheless, both critics and promoters of nuclear energy voiced negative and positive opinions: one group of critics requested that all copies of the booklet be destroyed, while another wanted 2,000 copies for local distribution and complained about the small number printed (15,000).

Votava

Chancellor Kreisky: his office organised campaign

The experts and organisers, at least the pro-nuclear ones, imagined that the public would listen eagerly and trustingly to the specialists' lectures in spite of strong fears about nuclear energy. The majority of anti-nuclear experts saw the audience as a concerned population with legitimate claims. The possibility of changes in the concept of the campaign as a result of effective, well-organised public action was ignored by the organisers as impossible to incorporate within the chosen framework. This indicated little flexibility in the organisation and an unwillingness to accept active public participation.

The main features of public reaction to the campaign became evident during the first public discussion in Vienna on 14 October 1976. There was strong resentment at the idea of listening passively to a panel discussion and only being allowed to submit factual questions in written form; the public demanded open discussion and it soon became apparent that the rules would be impossible to enforce. Massive distrust was expressed in the campaign: there was some surprise in the first debate when it became apparent that three of the panel experts were nuclear energy critics as opposed to mere propagandists.

Lack of consideration for the political viewpoint was another source of provocation to the public: while general problems concerning nuclear power were being discussed, Austria's first plant was nearing completion. The decision to build had been taken long ago and the construction proceeded throughout the campaign. Pressure was exerted to stop the plant going into operation and a resolution was passed to this end. Finally, the concept of having separate topics for each evening, in different towns, was criticised; although the theme of the first, general social and economic problems, was sufficiently broad, some of the subsequent ones were of little interest to the public which was anxious to discuss the more crucial issues.

In spite of the criticisms expressed during the first debate, plans for the next discussion in Linz remained unaltered; it was to be even more strictly controlled from the platform. The reaction was especially vehement, as the topic of the evening, energy policy, did not seem relevant to the people living near the proposed site of the second nuclear power plant. In addition, no outspoken critic was among the panel. Consequently, with almost unanimous consent, about fifty organised critics took over the panel and continued the meeting with their own chairman.

To reduce dissatisfaction with the division into different topics, it was decided to reinforce the panels with 'consultants', mainly experts in the biological and medical fields, who could be called upon if necessary. The next four debates proceeded uneventfully, even when two of the panels, dealing with economic problems, showed a strong pro-nuclear bias. The public discussions became less rigidly controlled and organisers and chairmen held preparatory talks with local opposition groups before each meeting, giving the latter the right to read longer statements or resolutions if they respected the rules of the debate.

The precarious balance achieved by these conciliatory measures broke at the seventh debate, in Vienna, on 27 January 1977. The hall could not seat all the people who arrived but after massive pressure short of violence, everyone was finally allowed in. The first panel statement, made in a provokingly patronising way by a promoter of nuclear energy, was halted by interruptions and a recognised critic among the panelists elected a new chairman. Thereafter, the discussion concentrated on how to stop the construction of Austria's first nuclear power plant.

The following two meetings, held in small provincial towns, were less dominated by the critics; these were comparatively peaceful, although the main criticisms were upheld. The final debate in Vienna, due on 24 March, will probably be controlled entirely by the opponents of nuclear power.

Coverage of the campaign by the mass media has been poor, being limited to superficial accounts of the arguments presented by the experts or the more spectacular actions of the protesters. Opponents have developed the theory that the government is deliberately hindering publicity in order to avoid raising public concern. Although radio and television are not state-controlled, it is indeed debatable whether sufficient efforts have been made to ensure adequate coverage.

At present, plans for the second phase of the campaign are in progress. Representatives of opposition groups, electrical utilities, the nuclear industry, as well as employers' and employees' organisations, will be invited to four symposia. These, to be held in May and June 1977, will be devoted to a discussion of the reports compiled by the ten expert groups. The results, together with a summary provided by the government, will be submitted to parliament, to serve as background for a subsequent decision on nuclear energy, and then published.

From a preliminary evaluation, certain points emerge:

- The organisers exercised considerable control, through their choice of experts, on the political credibility of the debate. But they had no influence over public reaction.
- Considerable differences emerged between public and internal debates. While the latter usually took place in a friendly atmosphere in which disagreements were respected by colleagues, opponents and proponents were extremely conscious of their respective roles in the public debates.
- The public was highly distrustful of expert panels set up by the authorities. There were accusations that experts were being paid by the nuclear lobby and that the government was staging a mere puppet show with political decisions having already been taken behind closed doors.
- The public reaction manifested 'scientific populism' and resentment of establishment science. Dissenting scientists quickly became celebrated as heroes. Although opposition groups sometimes displayed a moving willingness to delve into scientific details, anything outside their pre-conceived notions was unacceptable to them. What they demanded was a 'people's science'—a body of knowledge controllable by them.
- Coverage by the mass media was mostly determined by factors unrelated to the information campaign. It tended to dismiss the protest movement as extremist or based on irrational fears; opposition newspapers automatically wrote in a negative way about the campaign. Only in Vorarlberg, where the population had been made aware by opposition to a planned Swiss reactor close to the border, did the local movement receive strong press support.
- The authorities had not considered the political context sufficiently: the public was expected to consist of 'neutral citizens' only, to whom information would be offered as a public service. This neutral citizen, however, either doesn't exist, or is not sufficiently interested to attend the meetings. ☐

Traditionally, a large percentage of Austria's electricity requirement has been met by hydropower. The growing energy demands of the country's economy, however, finally led it to consider the nuclear option. In 1971 construction of the first nuclear power station began near Zwentendorf in Lower Austria. It is being built by the West Germany company Kraftwerke Union, although Austrian firms supply know-how and a considerable number of components. The company owning it is half state-owned and half-owned by the utilities in the federal provinces. The station should be supplying electricity to the grid by March 1978.

Public concern grew when talk began of a greater commitment to nuclear energy. The construction of a 1,300 MW plant was originally scheduled to begin in 1976; another was planned for the early 1980s. In the spring of 1975, groups of concerned citizens in Upper Austria —near the proposed site of the second plant—collected over 60,000 signatures in a local petition against the plans.

With nuclear energy a much debated issue in other countries and producing violent confrontations, with the recession (felt only slightly in Austria) reducing the need for greater electricity generating capacity, and with an election planned for the autumn of 1975 creating additional pressure on the government, all decisions concerning future commitments to nuclear power were postponed. Work on the planning of the second and third plants was suspended; construction of only the first plant proceeded normally. Later the government declared that the alternative to nuclear power would be increased use of (mainly imported) fossil fuels or imported electricity. In February 1977, responding to questions submitted by the opposition in parliament, the government pointed out that this alternative was economically feasible, although parliament would have to decide whether it was desirable. This decision is expected at the end of 1977. Of the three parties represented in the Austrian parliament, the governing Socialist Party is divided over the issue, but has a pro-nuclear bias, and the People's Party is pro-nuclear but highly critical of the way the government handles the problem: the smallest, the Liberal Party, is decidely anti-nuclear.

In the summer of 1976, the nuclear energy critics founded a nationwide federation outside existing parties incorporating eight local groups in all but one of the federal provinces. The number has since increased to at least twelve.

RADIOPHYSICS LIBRARY C.U.

Europe's nuclear debate (2)

Germany: growing acrimony

Werner Gries looks at the controversy over the use of nuclear power in West Germany

THE row in West Germany over the peaceful use of nuclear energy has grown increasingly fierce over the past few months. It reached its latest climax on 19 February with the siege of the nuclear power station at Brokdorf, where scores of people were detained by the police after a clash with militant demonstrators who tried to occupy the power station site. A similar demonstration last November, also at Brokdorf, was also violent. Meetings held all over the country to discuss the necessity for nuclear energy at all have produced clashes between supporters and opponents of nuclear power. The press, radio and television have busied themselves conducting detailed investigation into the pros and cons of the issue.

The result of all this activity, in some eyes at least, is little result at all. In spite of the wave of anti-nuclear demonstrations, a recent opinion poll indicates that a slight majority of the population is in favour of the construction of nuclear power stations. In an all-party resolution last year, the Bundestag (parliament) declared itself unanimously in favour of the continued use of nuclear energy so that West Germany's future energy supplies could be guaranteed; the Ministers President of the Länder (provinces of the federal republic) followed suit. Such agreement at government level, though, has not prevented various branches of the Social Democratic party calling for a halt to the building of nuclear stations; in some cases they have organised joint demonstrations with local people to protest against new plants. All political parties are involved in the debate, and each has in its ranks both supporters and opponents of nuclear power—the governing coalition of Social Democrats and Free Democrats displays more disagreement than the opposition Christian Democrats and Christian Socialists.

Federal complications

Additional complications have sprung from the country's federal framework. Individual Länder, for example, are responsible for authorising nuclear power stations in their own areas. Several local courts have granted injunctions to stop the building of new plants because of protests by private citizens. In Schleswig-Holstein, a court ruled that work on the Brokdorf nuclear power station could not start until a way of disposing of the waste had been agreed on, and in mid-February a court in Rhineland-Palatinate banned further work on the Müllheim-Karlich station near Coblenz because of technical errors in the construction. The government of North Rhine-Westphalia has banned the building of all new power stations within its area until a method of waste disposal is agreed. Some sites have been brought to a standstill, others have experienced partial stoppages; at some sites it has not even been possible to begin the construction work.

One of the major obstacles to have arisen to the expanded use of nuclear power has focused on the reprocessing and storage of radioactive waste. Although the importance of the problem was recognised when West Germany first began using nuclear energy, the necessary political measures to deal with it were not set in motion. What is more, after 1980 it may not be possible for West German fuel elements to be reprocessed in France or Great Britain. In both countries there is growing opposition to the expansion of reprocessing capacity, especially for foreign fuel. Germany's present contracts run out in 1979.

Faced with this problem, the West German government has resolved to authorise the building of new power stations only if the safe disposal of fuel elements can be guaranteed from the start. It has plans for a *entsorgunzentrum* ('service centre'), which could reprocess the fuel elements from all West German nuclear power plants and store the radioactive waste products in salt deposits for hundreds of years. The site chosen for the centre is Niedersachen, but a fierce argument has flared up locally which may have far reaching effects on the construction of nuclear power stations in other parts of West Germany. Yet unless the service centre can be established, the expansion of West Germany's nuclear power capacity will be halted. Government leaders and the opposition therefore feel that they must jointly take steps to ensure that a centre is built.

Overtaking Britain

At the end of 1976, 13 nuclear stations were in operation in West Germany, with a total output of 6,500 MW. West Germany has overtaken Britain in terms of nuclear power capacity and is the leading user of nuclear energy in the EEC. A further 17 nuclear power stations are proposed, some of which are already under construction. There were plans for 50 by 1985, with a total capacity of approximately 45,000 MW, but because of the recent public protests the construction of some planned nuclear power stations has been postponed in certain areas, and this figure now seems utopian. Experts now believe that about another 25 stations will be operational by 1985, with a total capacity of approximately 22,000 MW.

The government believes that the country faces an electricity shortage in the 1980s and in spite of the current difficulties, large scale research is going on to develop more advanced reactors. Some DM2,000 million each year are allocated for nuclear energy research, mainly for developing a fast breeder and a high temperature reactor. For many years there has been bitter argument over the necessity for the fast breeder, and this has grown even more bitter during the past few months. It remains doubtful whether an acceptable site can be found, and the special security problems make it more unlikely that the public will ever accept it.

Compared to the internal difficulties, discussion of Germany's role in international trade in nuclear technology has been comparatively limited. The controversial deal with Brazil, providing eight nuclear power stations, a reprocessing plant and an enrichment plant by 1990, was originally welcomed by all parties in the Bundestag, and the government and opposition still agree that it is necessary. The Carter Administration is objecting to the reprocessing and enrichment plants because it fears they will provide Brazil with the raw materials for an atomic bomb, and has endeavoured to pressure Brazil too. With reports now of US hold-ups in supplies of enriched uranium to West Germany, nothing is certain, but the general public seems to accept that the West German-Brazilian treaty should be adhered to in its original form. Their more immediate concern is with the domestic implications of a domestic programme. And that concern is likely to be fuelled still further in the future. ☐

Popperfoto

Demo at Brokdorf

UNITED NATIONS

Preparing for 1979

A Special Correspondent reports on preparations for the United Nations Conference on Science and Technology

THE appointment in January of Mr Joao Frank da Costa of Brazil as Secretary-General of the United Nations Conference on Science and Technology, which is scheduled for 1979, has enabled preparations to get under way. Sitting as the Preparatory Committee for the Conference, the 54-nation Committee on Science and Technology for Development gave a mixed reception to Mr da Costa's views on the organisation and timing of the conference preparations. He secured agreement, however, on several vital points, the most important of which is perhaps that he should have as free a hand as possible in the selection of the conference secretariat.

Kurt Waldheim, the UN Secretary-General, had stated that the post of Deputy Secretary-General of the Conference should be held by Dr Klaus-Heinrich Standke, the Director of the Office of Science and Technology in New York, and that his staff should form the core of the conference secretariat. From the first, Mr da Costa made it clear that he could not accept this arrangement, especially as Dr Standke was expected to double up two responsible jobs from now until the conference. No deputy as such has been appointed; the Executive Secretary of the Conference, Mr Hans Einhaus, who has lately represented the Office of Science and Technology in Geneva, is holding this position in an acting capacity.

Da Costa was less successful in persuading the committee of the practicability of some of his other ideas, such as the suggestion that each member state should produce three preparatory documents for the conference. If previous performance is anything to go by, many governments will have difficulty in producing a single really useful paper, as now requested, although the option to produce more remains open for those who can. Regional papers will also be prepared by the several UN Economic Commissions. With those from the other members of the UN system and from major organisations outside the UN, these will make up a considerable amount of documentation. To offset this, the number of meetings, seminars and so on suggested for the preparatory period will be reduced; the suggestion that the conference secretariat should be regionalised was also dropped.

No precise agenda is expected until this time next year, but the general objectives of the conference are already well defined. They include: the adoption of concrete decisions on ways of applying science and technology, as a strategy aimed at economic and social development "within a time frame"; strengthening the technological capacity of the developing countries; the adoption of means for the utilisation of science and technology in solving the problems of development; and the provision of "instruments of cooperation" to developing countries, for solving socio-economic problems that cannot be solved by individual action.

In general, it does seem as though the agenda will be action-oriented; one thing da Costa stressed was the need to avoid the "ritual complications" inherent in the preparations for all such conferences—the ritual of producing documents and of setting-up institutions do not of themselves solve problems, but are only tools to that end. In general, the objective of the conference will be to assess and redefine the role that science and technology has to play in development. Moreover, as seen by da Costa the preparations themselves can help towards the definition of a unified policy for science and technology within the United Nations system.

One other point on which da Costa did not get his way was in the location of the conference secretariat. He had asked that it be set up in Geneva, but this was overruled, the majority of delegations preferring New York, where they can oversee, if not interfere in, the conference preparations. Many regard this as unfortunate. One of the reasons for the success of the Environment Conference in 1972 was the insistence of Maurice Strong, as Secretary-General, that his headquarters should be in Geneva, away from the UN bureaucracy.

The New York siting could also prejudice really close collaboration on the part of the other members of the system, although their involvement has been one of the avowed intentions of those who suggested the conference in the first place. Geneva is central to these organisations—UNESCO in Paris, UNIDO and IAEA in Vienna, FAO in Rome and, in Geneva itself, WHO, WMO, UNCTAD and ILO. It is in these organisations that the bulk of international knowledge and experience in applying science and technology lies, and the possible economies of having the secretariat in New York may well be offset by a loss of their close collaboration and good will. □

CANADA

Support for alternatives

David Spurgeon reports from Ottawa on Canada's interest in alternative energy sources

CANADA, a country once thought to possess fabulous natural gas and petroleum supplies, is now carefully assessing its renewable energy resources. The government has recently increased the funds available for research on tidal power, wind power, biomass and solar energy. And the whole subject was highlighted at a recent meeting of the Parliamentary and Scientific Committee sponsored by SCITEC, the Association of the Scientific, Engineering and Technological Community of Canada. It was the second meeting of the committee, which was formed to bring parliamentarians and scientists together, and in spite of scepticism that this could be done, both meetings were well attended.

One of the most interesting energy potentials discussed was the Bay of Fundy's tidal power. Separating the two Maritime provinces of Nova Scotia and New Brunswick, the Bay of Fundy possesses one of the world's largest tide ranges. A. N. Karas, assistant director of planning for the Electrical Engineering Branch of the National Energy Board, said that although the last detailed study in 1968 concluded that Fundy power projects were uneconomic (albeit feasible), significant increases in power generation costs had helped the two provincial governments and the federal government to agree in 1975 to re-evaluate such schemes.

The conclusion was that the combined market for a 7,000 MW tidal power plant—in the Maritimes, in the contiguous areas of Canada (mainly Quebec), and in the Northeastern United States—would by 1990 be more than ten times the capacity of the plant, or about 77,000 MW, and that such a plant could be brought into operation by that time. A survey indicated that the anticipated peak load in the Maritimes would grow from 3,216 MW in 1980 to almost 23,000 MW by the year 2010. For Quebec, the peak demand would be 18,682 MW in 1980 and 133,000 MW in 2010. And in the Northeastern United States, the figures would be 18,000 MW in 1980 and 87,500 MW in 2010. Thus the full output of the plant could be used.

The energy displaced by a tidal power plant in the Maritimes, furthermore, would be predominantly high cost oil-fired and some coal-fired energy, the latter mainly in Nova Scotia. "In 1990 tidal energy would displace about 4,000 GWh of oil-fired generation and 500 GWh of coal-fired generation," Mr Karas said. "This is equivalent to about an annual savings of 6 million barrels of oil and 300,000 tons of coal. By the year 2010 the corresponding annual fuel savings have been calculated to be about 9 million barrels of oil and 600,000 tons of coal." To serve the tidal plant's export markets, extra high voltage transmission lines (735 or 750 KV) would be required.

The scheme has considerable appeal to residents of Nova Scotia, who have been hard hit by the recent increases in oil prices. Residents of that province pay some of the highest prices in the country for home heating. The president of the Nova Scotia Power Corporation, L. F. Kirkpatrick, was quoted in the *Globe and Mail* as saying that, based on recent construction cost estimates of between $1,300 million and $1,700 million, power could be produced at one or other of the Bay of Fundy sites for between 18 and 29 mills per kilowatt. Currently, oil-produced electricity costs almost 20 mills.

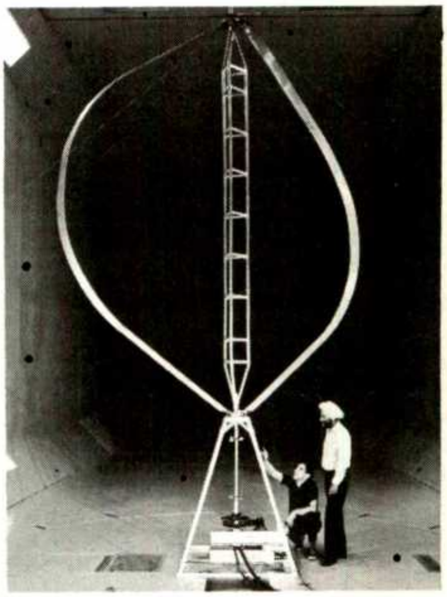
Dr E. P. Cockshutt, co-ordinator of the National Research Council of Canada's Energy Project, gave a cautious but positive forecast for the utilisation of solar energy in Canada. There is sufficient solar energy available in all the reasonably populated regions of Canada to make it a useful resource, he said, giving the lie to those who have played it down in the past. But there were unique features of climate and geography that necessitated a distinctly Canadian programme.

An attack on the technical problems of durability and cost was the most important priority for the next two or three years, Dr Cockshutt said, noting that "popular enthusiasm for solar heating presently outruns the technical and commercial realities." Large-scale commercialisation of solar heating is at least five to ten years away, but it is expected eventually to become a billion-dollar-a-year industry, he said.

Total radiation outside the earth's atmosphere available to a properly-oriented receiver amounts to about 1,400 watts m^{-2}, and that actually received on a horizontal surface at ground level in most of Canada is about one-tenth that (150 watts m^{-2}) on a year-round average basis, Dr Cockshutt said. Even the sunniest parts of the world receive less than twice as much, or about 250 watts m^{-2}. And with properly inclined collectors, the difference would be reduced. But it is because of the extremes of seasonal variation that a Canadian solar energy programme must differ greatly from those in countries close to the equator. In addition, the reflective properties of snow-covered ground differ distinctly from those of vegetation.

Professor H. M. Lapp, of the University of Manitoba's Department of Agricultural Engineering, called biomass a major energy source for Canada in view of the magnitude of its production annually. "The energy available in animal waste, crop residues and forestry waste represents approximately 6.72, 2.74 and 9.46% of Canada's national energy consumption, or a combined biomass energy potential of 18.92% of Canada's national energy requirement," he said. "Such a significant component of Canada's national energy requirements available from renewable sources should be examined carefully for the feasibility of economically viable development." Considering wood alone, he said it had been estimated that ethanol/methanol fuel production potential from wood was about 2½ times Canada's current annual consumption of motor gasoline and approximately 110% of its total gasoline plus fuel oil consumption.

The head of the NRC's low speed aerodynamics laboratory, R. J. Templin, estimated that, with average one- to two-mile spacing between megawatt-sized wind turbine power plants with a total height of about 100 m, the average wind power available over the whole of the country was about 130,000 MW (overall conversion efficiency 15%). If it was assumed that suitable sites were limited to areas within 150 km of an existing network, the available average power was reduced to about 60,000 MW—about twice Canada's national electricity consumption in winter months. Even without energy storage, "it appears that wind energy constitutes a very large *theoretical* resource in Canada," he said. The main obstacles to large-scale wind energy development were the need to demonstrate long-life reliability and to reduce capital costs.



Wind up: turbine in tunnel

A number of small-scale wind power projects are currently in progress in Canada, including several at the Brace Research Institute, Montreal, and a rotor designed by Brace installed by Hydro-Quebec's Research Institute for experiments on compressed air storage. The only large-scale project is the 200 kW vertical axis turbine now being erected by Hydro Quebec on the Magdalen Islands, one of the windiest sites in Canada. Several new versions of the turbine are under development at two Canadian companies with government funding assistance.

Only days before the SCITEC meeting, the federal government showed its interest in renewable energy resources by announcing that research in the area had been awarded the largest share of a $10 million increase in federal funding for 1977–78 for energy research. A Renewable Energy Resource Branch has been established within the Department of Energy, Mines and Resources, and a National Advisory Committee on Conservation and Renewable Energy is being created.

There was a $4.4 million increase for renewable energy research (including solar, wind, biomass and the use of heat pumps); energy conservation was allotted $3.7 million more; coal, heavy oils and oil sands research was increased $1.5 million; and transportation and energy transmission received an increase of $460,000. The increases will bring total federal energy R&D funding to approximately $138 million in the next fiscal year. □

VIKING

Give us a call, says NASA

Alex Dorozynski reports from Paris on the Viking symposium held there last week

NASA's next Mars package will be launched from Cape Kennedy in January 1984. It will carry a roving laboratory capable of covering at least 100 km and perhaps as much as 1,000 km of the planet. The orbiting satellite will contain 'penetrators', to be implanted at and to transmit information from selected locations, as well as remote sensing and transmission equipment. Lodged several metres into the Martian soils, the penetrators will record seismic activity and will try to determine the chemical composition of Martian soil below the surface. No attempt will be made to bring soil samples back to Earth; the earliest date for a rational sample return mission, rather than an indiscriminate 'grab sample' attempt, is 1988.

This schedule is not an official one, but it sums up the consensus of opinion at last week's symposium. The symposium involved senior scientific and management personnel of the Viking programme, the European Space Agency and the French National Centre of Space Studies. The Viking team will now tour several European countries, including the Soviet Union, to inform interested scientists of the latest Viking results and to emphasise that NASA planetary exploration programmes are open to international participation.

According to the Viking chief project scientist, Dr Gerald A. Soffes, no part of the schedule is in the approved stage. But the outlook is favourable indeed. By any standards—science, technology, public appeal—Viking has been an outstanding success, and data is still pouring in from the two landers and the two orbiters. These will all be kept at work for at least another full year. One single orbiter may continue to relay information from both landers, while the other will be switched over to different orbits in order to roam the surface of the red planet with its remote sensing equipment.

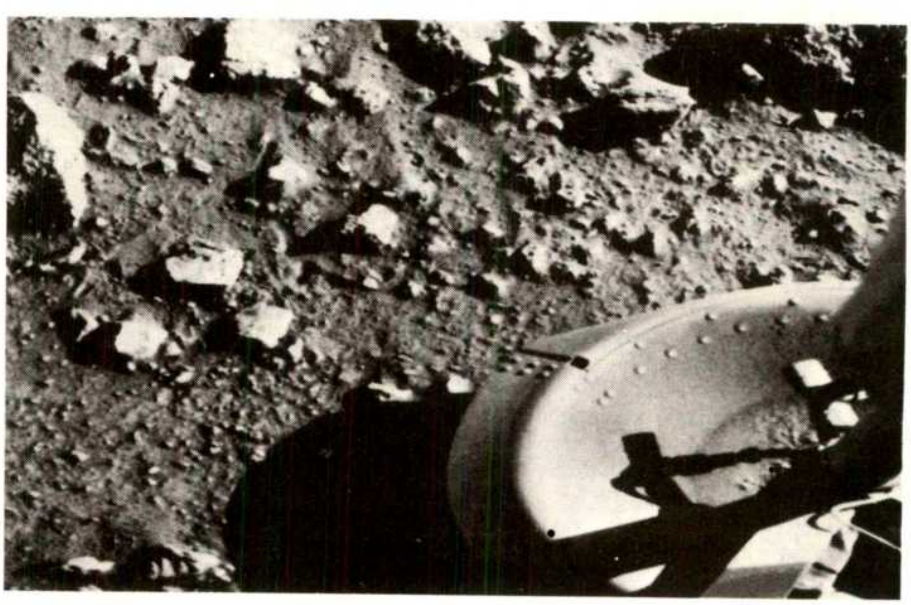
President Carter has recently proposed an increase in NASA 'study money' for 1978 (the increase awaits Congressional approval) but the agency does not seem to want to go to Mars with another Viking just for the sake of it; rather it wishes to exploit the massive flow of data to prepare the next Viking generation. The 1979 'astronomical window' to Mars seems too close for this, so the project for early 1984, even though it is not optimal from the energy viewpoint, has been adopted as a goal by the study team and this is already at work.

Dr Soffes and his colleagues made a point of inviting non-American guest scientists to participate in the study of Viking data and the elaboration of future missions. According to A. Thomas Young, the Viking missions' operations manager, participation would include the elaboration of scientific experiments, instrumental design, and perhaps even the loading on board of an experimental package—provided of course it fits into the overall package. Dr Young suggested that the first proposals be made to the NASA by mid-May this year, so that planning of the still-hypothetical 1984 mission could start early in October.

Meanwhile all Viking data is being made available to interested scientists. Major reports have been published in three issues of *Science*; these have not apparently been found sufficiently detailed and complete by some specialists, so a thorough review is now being prepared, including most recent data, for publication in the *Journal of Geophysical Research* in September.

Dr Soffes also hopes for an international Viking conference similar to the one that followed the Mariner mission, but, he says, "it is not easy to put together or to know when it should happen." In the meantime limited discussions on specific aspects of Viking results will be held during other meetings. These will take place, for example, at the lunar samples meeting in Houston in March, the meeting in Kioto in June of the International Biological Society for the Study of the Origin of Life, and the pulsar meeting in Tel Aviv, also in June. Scientists are also being encouraged to contact NASA directly concerning aspects of Viking in which they are particularly interested.

New data is coming in so rapidly that publications cannot keep up without delays of six months or more, and the participants in the Viking symposium at the *Palais de la Découverte* in Paris were given more than enough to whet their appetites. On the menu: the Mars quake, registered during the primary mission, which took place some 50 km away from the landing site, and was measured between 2 and 3 on the Richter scale; the first close range photograph of Phobos, one of Mars's small moons; the decreasing atmospheric pressure, now increasing again as winter approaches in the northern hemisphere; the verification of Einstein's general theory of relativity; the phenomenon of Mars' dusts storms and the unusual erosion patterns that have been photographed and still remain a puzzle. Finally, of course, there are the biology experiments, which Dr Leslie Orgel, Head of the Viking molecular analysis team, described as the most positively negative, adding that in fact they had given no answer; Viking had landed in one of the "most desolate positions on Mars". Other sites will be explored during the next mission; but Mars conditions, or at least some of them, are now being re-created in the laboratory to find out whether earthly life forms can survive in them. As other sites may have conditions entirely different from the ones at the Viking landing sites, there is still hope for life. "We may have found that where it's red, it's dead" quipped Dr Orgel; but Mars is red all over. ☐



NASA

Back to 1976 : a view from Mars

USA

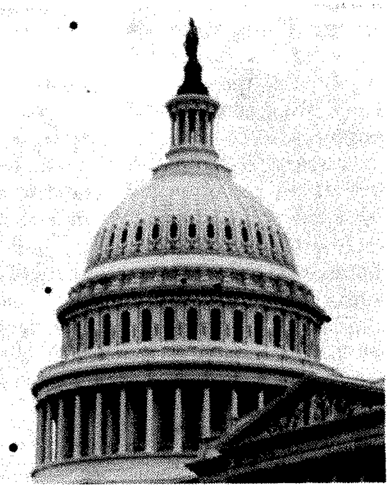
● Congress has already begun to whittle down the hefty budget increase for basic research proposed by President Ford in his valedictory budget. A key House subcommittee last week substantially reduced the budget request for the National Science Foundation (NSF), and recommended some shifts in priority among NSF's programmes. Though the sub-committee's actions are likely to be revised as the NSF budget bill moves through the rest of the Congressional budget process, the proposed reductions at least sound a warning that Congress isn't going to rubber stamp the research and development proposals this year.

Ford has recommended that basic research in general be given a budgetary shot in the arm to help restore some of the inflationary losses incurred during the past few years. It has been estimated that real support for basic research in the United States shrank by about 20% between 1967 and 1975, and Ford accordingly proposed that federal support be increased next year by more than 9% —well above the anticipated 6% inflation rate. NSF's budget, in particular, would have gone up by more than 12% under Ford's proposals. Those recommendations were left unchanged by President Carter when he submitted his budget revisions to Congress late last month.

Last week, however, the House subcommittee on Science, Research and Development, which is usually friendly toward NSF, approved an authorisation bill for the foundation which would provide an increase of about 9%—enough to keep NSF a step ahead of inflation, but substantially less than the Ford budget proposal. The subcommittee, moreover, has shifted funds within NSF's total budget in such a way that the programme of Research Applied to National Needs (RANN) would bear the brunt of the reductions, while the foundation's education support programmes would be given more money than Ford had requested.

The RANN programme would be given about the same overall budget as it received this year, but the subcommittee has recommended that a sharply increased proportion of RANN funds should be spent on earthquake research. Other RANN projects would consequently be cut back. Outside those two areas, the subcommittee has essentially recommended that NSF's programmes be given cost-of-living increases.

The subcommittee's action is only the first stage in a protracted budget process. The subcommittee bill will be taken up by the full Committee on Science and Technology this week before being sent to the House itself and, on the senate side, a subcommittee chaired by Senator Edward Kennedy has just completed hearings on NSF's authorisation bill; it is expected to report its own version soon. Appropriations committees in both the House and Senate will then take up the measure.



It is thus likely that the subcommittee's bill will be modified considerably as it progresses through the rest of the Congressional mill, but the proposed reductions nevertheless suggest that NSF's budget may be in for a bumpy ride through Congress this year.

● Another distinguished scientist has been appointed to a top-level post in the Carter Administration. Donald Kennedy, professor of zoology at Stanford University, was last week named Commissioner of the Food and Drug Administration (FDA), a post which has been occupied by physicians for the past 12 years.

The appointment is something of a surprise since Kennedy has had little direct experience in the political minefield of drug regulation. FDA is constantly caught in a fierce crossfire between the drug industry, consumer advocates and Congressional critics. It has been chronically short of funds to carry out its mission or regulating the powerful food, drug, cosmetics and medical devices industries, and its own research enterprise is generally acknowledged to be relatively low in quality. Kennedy will clearly have his work cut out for him.

A neurophysiologist, Kennedy's political star has been rising rapidly in Washington in the past few years. Elected a member of the National Academy of Sciences in 1972 at the age of 40, he was chairman of an Academy committee which last year produced a massive and widely-publicised report on pest control problems. Late last year he was appointed a senior consultant to the newly established Office of Science and Technology Policy (OSTP), a job which took up half his time and kept him shuttling back and forth between Washington and Stanford. While at OSTP, Kennedy is said to have played a major role in securing a substantial increase for agricultural research in President Ford's final budget. Otherwise, his experience in Washington politics has been limited.

● An international coalition of scientists, environmentalists, theologians and philosophers has been formed to seek a worldwide moratorium on most uses of recombinant DNA techniques and broader public discussion of the ethical and social implication of genetic engineering. The coalition, whose formation was announced at a press conference in Washington on Monday, includes among its sponsors Sir MacFarlane Burnett, an eminent Australian immunologist and Nobel Prizewinner. Other sponsors include Aurelio Peccei, founder of the Club of Rome, Lewis Mumford, author and philosopher, George Wald, and a number of environmentalist organisations and church leaders.

A statement endorsed by the group, which calls itself the Coalition for Responsible Genetic Research, notes that "genetic and molecular biological research is rapidly providing the basis for the practice of genetic engineering". It continues: "The continuation of this research without public understanding and approval, and in fact without a full comprehension of its potential by most of the involved scientists, poses a worldwide danger which is intensified by the fact that industrial investment in the developing genetic technology has already begun".

Citing possible health hazards from current research, including "the spread of cancer or the creation of new diseases", the statement calls for a moratorium "on all research that would produce novel genetic combinations between distant organisms which have not been demonstrated to exchange genes in nature". The moratorium should stay in effect, the coalition argues, while the ethical implications of the research are examined and the dangers assessed in public discussions.

Colin Norman

View and review

The development of a comprehensive UK energy policy has come in for further analysis in the past week. Chris Sherwell reports

BRITAIN'S Energy Secretary, Mr Anthony Wedgwood Benn, who has already taken steps to broaden the basis of consultation on the country's energy policy by holding an energy conference and proposing an energy commission, is now considering the possibility of publishing a Green Paper (discussion document) later this year to widen the debate still further. The suggestion is contained in a memorandum from Mr Benn which accompanies the energy policy review document that the Department of Energy has produced for the National Economic Development Council.

The document crisply summarises the country's prevailing energy policies. Short-term problems, arising from an excess of capacity, emphasise the difficulties of power plant manufacturers, the possibility of a coal surplus and the need for a gas gathering pipeline to harness the Northern North Sea. The suggested long-term strategy emphasises the Plan for Coal and coal conversion research, the need for a flexible oil depletion policy and a long-term gas policy, the need for access to necessary reactor technology including the fast breeder, and the benefits of conservation and renewable energy sources. The finances and pricing of the nationalised energy industries also need to be put on to a proper economic basis.

Last week the Royal Society released its long-awaited report on the government's discussion document published last year, *Energy R&D in the United Kingdom*. The Society also published a report on R&D strategy for offshore oil and gas technology. The most significant point to emerge concerned the fast breeder reactor. Coal could not alone satisfy future demand, it was argued, and the proposed demonstration commercial breeder (CFR-1) should be given sufficient approval to allow all planning and practical studies to start immediately; the country's lack of uranium sources meant that any credible nuclear policy had to be based on the breeder and did not imply the inevitability of a breeder programme.

Support for R&D on alternative sources was urged, particularly in power and solar power; wind and geothermal energy were less enthusiastically received. ☐

THE severe drought in the Western United States has led to widespread calls for rain- and snowmaking, and has even caused one state, Idaho, to threaten legal action if a neighbouring state, Washington, interferes with the weather. Is there, however, any reason to have much confidence in rainmaking activities? Many of those who work in or close to the field of weather modification are still sceptical of our ability to affect precipitation.

Most effort in weather modification has been devoted to adding to the population of natural ice nuclei. These are extremely rare particles (only about one in a billion atmospheric particles is an effective ice nucleus at warmer than −15 °C), although these rare nuclei can sometimes generate many secondary particles. Silver iodide is still the main artificial nucleating material, although experimental evidence on the mechanism is ambiguous. Distinct from ice nucleation is the process of altering populations of cloud condensation nuclei, and some interesting work has been done in India using sodium chloride. There is still no strongly preferred method for launching nuclei—airborne and ground generators continue to be used.

An immense amount of cloud-seeding has been done in the past 25 years, but most has been by commercial enterprises for which little satisfactory documentation exists. Detailed studies by governmental organisations with attempts at statistical analysis have, however, been conducted in the United States, Canada, India, Israel and Australia and are summarised in a valuable document, *Weather and Climate Modification* (US National Academy of Sciences, 1973).

Changing weather

BACKGROUNDER

Experiments have used amounts of silver iodide of up to 1 kg per hour. The major problem has always been that of data analysis, as ideally, trials should be fully randomised. Even when this has been done statistical tests, on which there is still much discussion, show occasions when results are inconclusive and even occasions of diminished precipitation. The above-mentioned report, however, was more optimistic about one experiment, Climax in Colorado, in which cold orographic winter clouds were seeded; precipitation, it says, can be increased by substantial amounts (35%) and on a determinate basis.

Not all weather modification experiments have been specifically devoted to rainmaking. In Florida, a major experiment has been underway for several years in the field of 'cloud management'. Massive seedings of up to 1 kg per cumulus cloud are intended to release large amounts of latent heat as supercooled water is rapidly turned to ice. The latent heat increases the buoyancy of clouds and hence their lifetimes during which they may grow or merge.

Further, there has been extensive activity, centred on Colorado, to modify hail which accounts for $700 million of damage in the United States every year. A strategy already adopted in the Soviet Union is to increase by a factor of up to a thousand the number of nuclei in the hail-producing part of a cumulonimbus cloud by launching shells or rockets packed with silver iodide which explode inside the cloud. The hope is that many small hailstones rather than a few large ones will fall. Soviet work has extended well beyond the research phase and annual savings running into tens of millions of roubles have been reported. Work in France, Switzerland and Kenya has yielded mixed results, again with problems of randomisation, but the National Hail Research Experiment initiated in 1971 in the United States has yet to bear fruit.

IN BRIEF

More trouble for dissidents

Seven Moscow Jewish activists, all members of the Voronel/Azbel' seminar and/or the Helsinki monitoring group, have had their homes searched unusually thoroughly by the police. The seven are the cyberneticist Aleksandr Lerner, Josif Beilin, a mathematician, Ida Nudel, an economist, and the engineers Vladimir Slepak, Anatolii Shcharanskii, Boris Chernobil'skii and Mikhail Kremen. An open letter in *Izvestiya* signed by Dr Senya Lipavskii, a former associate of these groups, accuses them of being financed by CIA funds. Lipavskii also claimed that during their protest hunger strike of March 1974, the sinologist Vitalii Rubin and the chemist David Azbel' did, in fact, eat regular meals. (Both Rubin and David Azbel' have since emigrated, Azbel' in 1974 and Rubin in 1976).

Dissident circles in Moscow associate the new repressions with a recent article in *Pravda* urging political vigilance "as never before". In Warsaw, meanwhile, the latest batch of arrests of members of the Workers' Defence Committee has included the mathematician Jan Lytinski and Mieczyslaw Grudzinski, a computer engineer.

Argentina–Peru deal

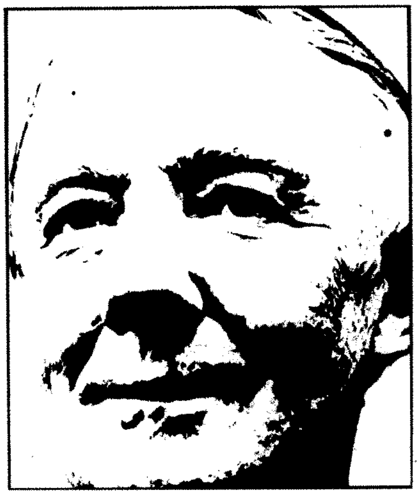
Argentina's growing role in Latin America's development of nuclear power was given another boost at the end of last week when she signed an agreement with Peru to build a 10 MW research reactor there. The agreement, reportedly involving a $50 million budget, covers design, equipment and training of local scientists. Argentina herself has a 300 MW reactor in operation, and a 600 MW reactor under construction. Recently she announced her support for Brazil's efforts to maintain the controversial deal with West Germany, the original source of guidance for her own programme.

BNFL: another step

Plans submitted to Cumbria County Council by British Nuclear Fuels Ltd (BNFL) for expansion of existing Magnox reprocessing facilities and for facilities to develop its Harvest waste solidification process were, as expected, quickly accepted by the council's planning committee last week.

BNFL split into two its more controversial oxide fuel reprocessing plans. The part covering construction of the plant is now expected to be called in for a public inquiry. The other part, relating to the provision of storage facilities for incoming fuel, will, BNFL says, not prejudice any decision on the plant itself.

Grade expectations



KENNETH MELLANBY

SCIENCE is being taught today in British universities, polytechnics and colleges in many different ways. We still have special honours degrees, where one subject is dealt with 'in depth', with the intention of producing specialists. It is generally agreed that brilliant students, particularly if they intend to follow a career in their speciality, may thus be well served. However, some departments now offer such a variety of optional courses that the result may be unrecognisable to the traditionally minded. Zoologists may never study a whole animal, botanists may know little about common plants. Even when such fashionable absurdities are avoided, many would agree that less gifted students, only able to scrape a pass, are better served by more broadly based instruction.

The 'interdisciplinary degree', however, is not new. Fifty years ago Part One of the Cambridge Natural Science Tripos (taken in three subjects), or the similar London University General Honours course were familiar examples; both entitled their holders to an honours BA or BSc. The degrees were considered suitable for the less able, or as a preliminary to specialisation in a single subject.

Today, there is a much wider choice. Modular degree courses may allow students to choose combinations of subject which appear illogical to conservative academics. Even when, as is true of most colleges, students are sensibly counselled, so that the subjects studied are reasonably compatible, those entering these courses may have somewhat doubtful job-prospects, particularly serious at a time of graduate unemployment. It would be little consolation for a prospective chemist to be told that he is now better suited to enter "the upper reaches of the Civil Service, banks, the highest positions in industry" because he is now better educated than his contemporaries studying classics (if his course fits the criteria suggested by Sir Hermann Bondi, *Nature* 27 January 1977) when the Royal Institute of Chemistry finds itself unable to recognise his qualifications.

This was why the Council for Science and Technology Institutes, which coordinates the work of the recognised bodies in Britain, held a meeting in London recently to discuss how multidisciplinary degrees could qualify their holders for admission. It was clear that all the institutes were anxious to find ways to allow these graduates to qualify, provided this did not lower their standards. Most give credit for those parts of the course which are relevant, and suggest how essential gaps may be filled. As all the institutes only elect graduates after several years of experience in their subject, the length and nature of this experience can be adjusted to overcome deficencies. It is clearly important that all teaching departments should know, in advance, how new courses are likely to be viewed by the professional institutes, as well as by prospective employers.

Membership of professional institutes may become increasingly important, as these qualifications become the means by which scientists are recognised within the Common Market. It is also clear that the greater difficulty a candidate may have in being accepted the more valuable membership will be to him. The institutes must be fair and sympathetic in all cases, but must also be seen to be maintaining their standards. They must always remember Groucho Marx, whose applications to join an exclusive club were repeatedly blackballed. Eventually he was informed that he had been successful, upon which he asked "Who would want to join a club which admitted a bum like me?"

correspondence

The teratogenic effects of dioxin

SIR,—Alistair Hay (10 February) states that many pregnant women from the dioxin-contaminated district of Seveso have sought terminations outside Italy. Some will have to come to Britain: UK statistics should reveal whether relatively more abortions were performed on Italian women in 1976 than 1975.

In view of the highly teratogenic activity of dioxin in animals, and the total absence of information for our own species, I share Dr Hay's concern that no organised steps have been taken to examine aborted foetuses for possible malformations. I would urge that some code of practice be agreed, if possible for Europe as a whole but at least for Britain, to ensure that all foetuses aborted as a result of any suspected contamination of the environment should be examined by an experienced pathologist and compared with a matched control series. We may be too late this time: but Seveso is unlikely to be the last accident of its kind.

Yours faithfully,
ANNE MCLAREN

MRC Mammalian Development Unit,
University College, London

Journal abbreviations

SIR,—I agree with Adrian Smith (10 February, page 492) that the saving in typesetting does not in itself justify the use of journal abbreviations. I suggest, however, that the real problem lies not in abbreviating but in establishing a correct and unambiguous title. Here the British Standard is of no help, but the *World List of Scientific Periodicals* is invaluable, for journals published up to 1960.

The examples of ambiguous abbreviation cited by Mr Smith do not come from the *World List*, where there is a clear distinction between *J. math. Phys.* and *J. Math. Phys.* Had they existed in 1960, *Industrie minérale* would have become *Industrie minér*, while *Industrial Minerals* became *Ind. Miner.* Even in the annual supplements published since 1960, which have unfortunately abandoned the principles of the fourth edition of *World List*, *Ind. Miner.* (*Fr*) is not the same as *Ind. Miner.*

The *World List* gave a standard for journals with multilingual titles, and distinguished, if distinction be thought necessary, between three journals called *Nature*.

Correct identification of current journals will eventually be possible by the use of International Standard Serial Numbers, though whether this will reduce the chore to authors and editors remains to be seen.

Yours faithfully,
J. E. M. HORNE

Freshwater Biological Association,
Windermere

Charged plumes

SIR,—Contrary to the report of Jennings and Jones on high electric fields from industrial stack plumes (November 18, 1976), the electrical effects in the atmosphere caused by electrostatic precipitators are well known. Those working in the field of atmospheric electricity are aware that plumes emanating from chimneys equipped with precipitators contain large numbers of charged particles which can be detected at a considerable distance from the chimney[1].

Much of the charge may be carried on solid particles which somehow have escaped capture on the precipitator surfaces. We think that some fraction of the charge is also carried by water molecules from the combustion products in the industrial operation and of course there will be some small ion generation from the breakdown of other gases near the high voltage portion of the precipitator.

We have measured effects of chimney plumes with equipment carried in a manned aeroplane[2,3,4]. Among other results, we observed both the horizontal and vertical atmospheric electric field components up to 4,000 feet above the chimneys on calm days, when thermal convection of the plume was more pronounced than motion downwind. Rowland[5] came across the plume phenomena in 1973 when making measurements in a clear air turbulence study.

Vonnegut *et al.*[6] purposely generated clouds of charged oil droplets with a device similar to an electrostatic precipitator and reported extremely high local electric fields which caused point corona discharge currents and other high-field effects on bushes, structures, people, etc. He also levitated a charged cloud of water droplets below the oil cloud.

To the extent that charges generated in this manner (by chimney precipitators) can somehow become transferred onto high mobility carriers, the authors may be right in asserting that there is a possible contribution to the earth's charge balance. We doubt that the low mobility, charged solid particles contribute directly, but the high mobility charges which they may generate by pulling point discharges from earthed structures, might. The first result of this point discharge current in the atmosphere, of course, will be to help discharge the chimney plume; no doubt this is partly why the electrical effect of the plume is diminished as it travels away from the precipitator.

The observation of a possible hazard from sparking in the near vicinity of such plumes is well taken, and the cement and electrical power industries should be interested in further investigation into this likelihood.

Yours faithfully,
MAYNARD L. HILL
THEODORE R. WHYTE
ROGER O. WEISS

Applied Physics Laboratory,
Johns Hopkins University,
Laurel, Maryland 20810, USA

[1] Moore, A. D. *Electrostatics and Its Applications* 402 (John Wiley & Sons, N.Y., New York, 1973).

[2] Hill, M. L. & Whyte, T. R. "Investigations Related to the Use of Atmospheric Electric Fields for Aircraft and RPV Stabilisation," APL/JHU TG 1280, June, 1975.

[3] Whyte, T. R. "Atmospheric Electric Field Research Program—Preliminary Report for April, May and June, 1975." APL Report BAF-75-07, July, 1975.

[4] Hill, M. L., Weiss, R. O. & Whyte, T. R. "Investigation of the Use of Atmospheric Electric Fields as a Vertical Reference for Airborne Vehicles, Progress Report #3." APL Report BAF-76-20, September, 1976.

[5] Rowland, J. R. "Airborne Measurements of the Atmospheric Electric Potential Gradient Associated with Haze, Smoke Plumes, and Clear Air Convection." APL Report MPD73U-032, 3 May, 1973.

[6] Vonnegut, B., Smallman, C. R., Harris, C. K. & Sykes, W. G. "Technique for the introduction into the atmosphere of high concentrations of electrically charged aerosol particles." *Jour. of Atmospheric and Terrestrial Physics* **29**, 781-792, 1967.

Bird at Pompeii

SIR,—T. E. Thompson (27 January, page 292) surely owes an apology to the anonymous artist of Pompeii. The little bird he portrayed sitting on a rock overlooking the sea is clearly a composite representation of all those passerine migrants of the type known to Americans as 'confusing fall warblers' which are still trapped and eaten on a vast scale all round the Mediterranean coastline. It is interesting that they are the only terrestrial delicacy considered worthy of inclusion in a picture of local specialties. They are of course those 'nightingales' whose tongues formed the favourite diet of the Roman emperors.

Yours faithfully,
W. R. P. BOURNE

Milltimber,
Aberdeen, UK

news and views

Unsolved problems in nuclear structure

from Otto Kofoed-Hansen

IN preparing the balance sheet for any particular branch of science, much subjective argument is involved. So what follows is my very personal stocktaking of nuclear physics—where do we need more experimental information?

Where exactly is the yrast line?
Quoting Bohr and Mottelson (*Nuclear Structure* **II**, Benjamin, 1975) "the lowest level of given spin is referred to as an "yrast" level (Grover *Phys. Rev.* **157**, 832; 1967) The word appears to derive from the old Germanic *wor*, which developed into the English *weary* and the Nordic *ørr* (modern Swedish *yr*, modern Danish *ør*), meaning dizzy. The form *yrast* is the naturally constructed superlative of *yr*" (In other disciplines of physics the name "the lowest Regge trajectory" appears more natural)

For a given nucleus composed of Z protons and N neutrons, (mass number $A=Z+N$) there exists a multitude of possible states. The states are characterised in their energy content and in sets of quantum numbers (such as spin, I, which characterises the angular momentum of the nucleus as a whole) For each spin value, I, there exists a lowest energy content below which no (N,Z) nucleus of this spin can be formed. This lowest energy limit is called the yrast line—at this line all the energy available is used to make the nuclear structure in question spin. For simple heavy nuclei there is also a maximum energy content beyond which the nucleus will fission, centrifugal forces will split it. Thus there exists for all NZ combinations and all values of I a region limited on one hand by fission and on the other hand by yrast (dizziness). Large groups of nuclear physicists are attempting to pin down these limits and to understand them in terms of nuclear models. The tools envisaged are heavy ion accelerators and all possible refinements in detector techniques The aim is to create very large spin states of representative combinations of N and Z and to watch the details of the decays of these states The mere fact that N, Z and I can vary within a relatively wide range means that this is a vast region of research. The interpretation is forseen in terms of collective nuclear models, but bordering on those limits where every single nucleon in the nucleus carries as much oriented angular momentum as it can get away with. The next question thus follows quite naturally.

Where is the borderline between continuum descriptions and individual particle descriptions of nuclei?
By 'individual particle description', I understand a description where the nucleus of mass number $A=Z+N$ is considered as a many-body system of A interacting nucleons (Z protons and N neutrons). This should not be confused with an 'independent particle description', where a nucleus is described as usually one (or a few) particles moving in an average field created by the rest or by the totality of nucleons in the nucleus and described by semi-empirical parameters. Whereas I personally consider an "individual particle description" as a fundamental approach, I must admit the technical difficulties encountered. For practical purposes I am quite willing to use the semi-empirical approximations. I have, however, a (vain?) hope that some day we may succeed in using the fundamental approach. It is clear that the yrast region is such a border line, because when this region is reached the nucleus spins as fast as possible with a finite number of constituents having oriented angular momenta. But are there other ways of assessing such limits?

What is now the major difference between the results obtained with a genuine many-particle description and with a continuum model? To my mind the major difference is to be found in the number of levels of this or that class which obtain An example of this can be found apparently in the number of levels in a rotational band. A few years ago an attempt was made to answer such questions. I feel that it would be interesting to pursue this matter in much greated detail. Results of this kind would also be of interest for the subject of the next question.

Where do the mesonic degrees of freedom come in?
Perhaps this question may be most easily discussed by considering a few historical facts. After the Second World War nuclear physics had become an 'important science'. Many physicists were optimistic that the ultimate solutions to problems in nuclear physics would soon be found. One such attitude was a widespread hope that bombarding hydrogen with protons and neutrons of energies up to a few thousand million electron volts would provide a complete understanding of nuclear forces. Thus the field of high energy physics flourished with man-made fast projectiles joining cosmic rays as tools.

However the world of minuscule particles is much more complicated than imagined. It turned out that elementary particles appear in large families, that nucleons exist in excited states, that they have structure and internal degrees of freedom.

In the picture of nuclei composed of nucleons these degrees of freedom are neglected This is a parallel to the fact that when discussing molecules the internal degrees of freedom of the nuclei are usually neglected My question is really a query into the limit of accuracy to which it is permissible to disregard the mesonic or quark degrees of freedom. Last year the School of Nuclear Physics held at the Ettore Majorana Centre of Scientific Culture at Erice, Sicily, and directed by Denys Wilkinson, was exclusively devoted to this question.

The exotic degree of freedom called strangeness (the characteristic quantum number of kaons and hyperons) has been clearly identified in nuclear matter, forming the field of study of hypernuclear spectroscopy. How soon before we see charming nuclear spectroscopy? Thus there exists some knowledge of the level at which the effects appear. Similarly, excited nucleons (with strangeness zero), the so-called N*'s, have been considered in nuclear matter in the same way as they are considered in hadron–nucleon collisions, that is, as resonances, but now broadened by Doppler motion. But my question should really be reformulated: when N*'s, hyperons and so on are produced on nuclei is a nucleus then seen as a collection of quasifree nucleons, or can the effects of the nucleon binding, of the collective of particles forming the nucleus, and of the presence of pions during those exchanges that are responsible for the nuclear forces be discerned? Formulated in this way, it is evident that we ask questions which must be answered by the selection of rare situations or by very high precision. So far, the general feeling is that we have not succeeded in finding the answers. There has been a puzzling result, however.

Collisions between fast nucleons take times T_R of the order of R/c, with R being the nucleon radius in question and c the velocity of light, whereas experiments with fast projectiles take times T_L of the order L/c, where L is the length of our apparatus; the ratio of these time-scales is $T_R/T_L \simeq R/L \simeq 10^{-14}$ to 10^{-17}. Thus what is seen is something that has been fully developed in the apparatus, but may have very different aspects over periods of time that are but a few times T_R. Here the nuclei may be used as detectors enabling observations over nuclear distances R_N in the range $R < R_N < 6 \cdot R$ to be made Developments in ordinary material extending over metre distances can be called 'macroscopic' and developments over nuclear dimensions and with nuclear densities 'microscopic'. The three main results may be summarised as follows (see for example Cronin *et al. 1976 Tbilisi Conference on High Energy Physics*).

Macroscopically, cascades of particles produced near the forward direction are found, that is the incoming hadron produces fast particles, which in turn produce fast particles and so on; microscopically, no such cascading results.

If the final state is a 3π or a 5π state, that is three pions produced or five pions produced, then macroscopically the chance of collision (the cross section) is respectively three times and five times that of a single pion; microscopically, the cross section is very closely equal to that for one pion in both cases.

When very large momenta are carried over in the collision (that is, when the struck particle gains a large amount of energy), a relatively rare event has occurred. Naively, it would be expected that the chance of such events occurring in nuclear matter would grow with nuclear mass number as the nuclear cross section, that is roughly proportional to $A^{2/3}$. Experimentally, the result is proportional to $A^{4/3}$.

These three main results need explanation and further study both experimentally and theoretically. It is not known whether they are nuclear or hadronic effects.

All this does not answer the central question: how many degrees of freedom should we consider in nuclear matter? And when are we to talk about a finite number of constituents and when about continuum? These problems are also linked to the next major point.

Are shock waves observed in nuclear matter?

Certain authors maintain that they have seen such waves but for me the interpretation is not clear. First, I have

Variable DNA repeat lengths

from Rosalind Cotter

AFTER the revelation of the chromatin beaded structure there followed much tangled discussion about the amount, if any, of string between the beads, which was based chiefly on the different unit sizes generated by micrococcal nuclease in Oregon and Cambridge. The argument has now moved from the level of technical disagreements involving digestion termination conditions or faulty calibration to a careful comparison of DNA repeat size in different types of chromatin. A correlation with genetic activity is beginning to emerge.

Chambon and coworkers have examined the DNA repeat length of subunits in chromatin from higher eukaryotes (*Proc. natn. Acad. Sci. U.S.A.* **73**, 4382; 1976). Cells of mature tissues have nucleosomes containing 196 base pairs of DNA, genetically dormant cells rather more, and cells from actively dividing tissues rather less. It has been suggested that smaller DNA repeat lengths are found in primitive eukaryotes, the value for yeast being about 165 base pairs (Thomas & Furber *FEBS Lett.* **66**, 274; 1976), for *Aspergillus nidulans* 154 base pairs (Morris *Cell* **8**, 357; 1976), while 170 base pairs are found in nucleosomes of *Neurospora crassa* (Noll *Cell* **8**, 349; 1976).

These and more recent studies (Lohr *et al. Proc. natn. Acad. Sci U.S.A.* **74**, 79; 1977) have shown that, at early times of digestion with micrococcal nuclease, the most resistant portion of the repeating unit always has 140 base pairs. These core particles, formerly known as monomers, do not vary among species or from tissue to tissue (Morris *Cell* **9**, 627; 1976) with respect to size, histone content (two each of H2A, H2B, H3 and H4), and internal cleavage pattern by DNase I. The variations in repeat lengths are therefore attributable to the more nuclease-sensitive DNA spacer between core particles.

Lohr *et al.* observe a distribution of repeat sizes for yeast and HeLa chromatin, ranging from 140 (that is, no spacer) to 165 base pairs in yeast, to 190 base pairs in HeLa. Chicken erythrocyte oligomers however quickly reach constant sizes during digestion which are compatible with a regular array of core particles and 60 base-pair linkers. The authors speculate on a possible correlation between spacing mode and gene activity, which would be expected to be more complex along chromatin from active cells with sizeable transcribed and non-transcribed regions, and more regular in inactive chromatin in order to meet packaging requirements more efficiently.

The Cambridge group suggest that variations in spacer length may be brought about by histone H1. H1 is highly basic and is thought to interact with phosphates of DNA in the spacer regions. H1 from *Aspergillus* has fewer basic residues than *Neurospora* H1, and can therefore presumably protect only a shorter piece of interbead DNA. Morris notes a subunit repeat for chicken erythrocyte chromatin which is longer than that of chicken liver chromatin, and might be attributable to the partial substitution in the former of H1 by the more basic H5. He discusses the interesting possibility that specific recognition sites such as promoters might have altered accessibilities depending on whether they are incorporated into nucleosome cores or spacers; if then nucleosomes are phased with respect to recognition signals, this phasing could be altered by different H1-like proteins inducing changes in linker length. H1 is known to vary during development and cell differentiation and may therefore influence the accessibility of recognition sites in active chromatin.

no clear picture of what is understood by ordinary sound waves and the velocity of sound in nuclear matter. Bohr and Mottelson (*op cit.*) point out that in nuclear matter one must assume that one deals with zero sound, that is, vibrations that are close to collision-free. If this is the case, then the velocity of sound is likely to be relatively high and shock waves may need even more violent initiation than could first be imagined. To me, the domain is far from exploited and merits much further study both conceptually, theoretically and experimentally.

In addition to the questions raised above, much work needs to be done on nuclei far from stability, including heavy elements and the possible super-heavies. Exotic atoms also present an interesting field; with antimatter available, they can even be envisaged as A_1–$\bar{A}_2$ atoms with strong interaction complications.

All this is, of course, mainly academic. The practical nucleus remains. For the time being however, nuclear applications have become "despised and rejected of men". □

Acid proteases come of age

from Jordan Tang

In the past 10 years, research into the structure and function of proteases has been successful. The exception has been in the area of the acid proteases which has had the status of a stepchild—mainly because of the absence of structural information. But it now seems that the acid proteases have come of age.

Last November, at the 'Conference on Acid Proteases: Structure, Function, and Biology', held at the University of Oklahoma, three new high-resolution crystal structures of microbial acid proteases were reported. The amino acid sequences of two gastric carboxyl proteases, pepsin and chymosin (B. Foltmann, unpublished data) were completed, and a microbial enzyme, penicillopepsin, is near completion These results have provided a new framework for the study of the catalytic mechanism and the evolution of this group of enzymes. (The proceedings of the Conference will be published as a book in the autumn of 1977).

Among this group, the best combination of X-ray and sequence data is in the acid protease from *Penicillium janthinellum*· penicillopepsin This collaborative work of two Canadian groups is reported in this issue of *Nature* (Hsu *et al.* page 140) The enzyme is bilobal and has a pronounced cleft between the two lobes. The 2.8 Å resolution electron density map was calculated, using eight heavy-atom derivatives Although the amino acid sequence of this enzyme is not completed, the sequence of 16 fragments can be aligned against the known sequence of porcine pepsin (Tang *et al.*, *Proc. natn Acad. Sci. U S A* **70**, 3437, 1973). From this alignment, the polypeptide backbone and the side-chains of 326 residues can be traced in the electron density map with little ambiguity. The crystallographic data of acid proteases from two other moulds give essentially the same secondary and tertiary structures. The enzyme from *Rhizopus chinensis* has been solved at 3 Å resolution by D. R. Davies's group at the US National Institutes of Health and the enzyme from *Endothia parasitica* has been solved at the same resolution by T. L. Blundell and coworkers at the University of Sussex. Although little sequence information is available for these two enzymes, the tracings of the polypeptide chains are quite certain The folding of the chains are the same in all three enzymes and the secondary structures consist of large numbers of β-sheets with only a few isolated helical turns. The apparent binding clefts are also extremely similar In all three cases, the clefts extend across the surface of the molecules and are sufficient to accommodate the binding of about 8 amino acid residues of the substrate as suggested from the specificity studies of microbial acid proteases and pepsin

A close relationship of tertiary structures of porcine pepsin and the microbial acid proteases is indicated by sequence homology. As mentioned above, the fragments of penicillopepsin can be aligned against the pepsin sequence. Computer programmed comparison shows 11 highly conserved regions, including the regions near the catalytic aspartyl residues which will be discussed below The crystal structure of porcine pepsin is still uncertain. The previously reported 2.7 Å resolution structure (Andreeva *et al. Dokl Akad. Nauk U.S.S.R.* **228**, 480; 1976) shows a considerable number of contacts along the molecular boundary; a revision of the molecular boundary, as well as a new interpretation of this map, is underway. However, a comparison of the tertiary structures of pepsin and the acid protease from *R. chinensis* by Andreeva (unpublished results), using the data of Davies with the calculation of rotation function, indicates a high degree of similarity—confirming the implication of sequence comparison.

There has never been any doubt that the active centres of all the acid proteases are very much alike. Two active centre aspartyl residues, 32 and 215 in pepsin were identified by means of active site directed modifying reagents—an epoxide and a diazo compound. These reagents also inactivate most of the other acid proteases. In the crystal structure of all three microbial acid proteases, these aspartyl residues are found to be close to each other at the centre of the apparent binding cleft. In the case of penicillopepsin, the chains of Asp-32 and Asp-215 are in intimate contact so that, as suggested by Hsu *et al.*, they may share a common proton between the oxygen atoms of the two aspartyl residues. In addition, Ser-35 is hydrogen bonded to the other carboxyl oxygen of Asp-32. These three hydrogen-bonded residues. Ser-35, Asp-32, Asp-215, are reminiscent of the charge relay system of the serine protease All three residues are also conserved in the known sequences of acid proteases. The hydrogen bondings could easily explain the low *p*Ka of Asp-32 (~ 2) and the slightly high *p*Ka of Asp-215 (~ 4.5).

But, despite these new findings, the detailed catalytic mechanism of acid proteases is unlikely to be solved quickly. First, direct evidence on the position and orientation of substrates or products in the crystals, which undoubtedly will be studied soon, has not been obtained. The possible roles of some groups near the catalytic site (for example Thr-216 and Gly-217) are also uncertain. Second, although the suggestion of the catalytic roles of two aspartyl residues can easily be fitted to the crystal structures, this evidence does not exclude any of the previously proposed mechanisms based on the catalysis by two carboxyl groups. For example, the controversy regarding the presence of an enzyme–amide intermediate in the catalysis of acid proteases is still unsettled.

The binding position of pepstatin, a strong inhibitor for all acid proteases ($K=10^{-10}$ M for pepsin), has been determined at low resolution in the crystal structure of the *R. chinensis* enzyme (Subramenian *et al. Biochem. biophys. Res. Commun.* **68**, 875; 1975). This hexapeptide binds as an extended chain in the centre of the apparent substrate binding cleft. This finding agrees with the results of kinetic studies, using pepstatin fragments, which suggest that pepstatin is a transition state analogue of peptic hydrolysis (Marciniszyn *et al. J. biol.*

Chem. **251**, 7088; 1976).

The mystery of the mechanism by which the active centre is involved in the intramolecular activation of the zymogens of acid proteases seems to have been intensified by the knowledge of the tertiary structures. No zymogen has been found for the microbial acid proteases, but based on sequence comparison the NH_2-terminal position aligns closely in the linear structure with that of pepsin. Assuming that the NH_2-terminal chain in pepsin is positioned in its tertiary structure like those chains found in three microbial enzymes, then this polypeptide chain would be extended away from the active centre with the NH_2-terminus on the other side of the molecule away from the binding cleft. However, kinetic data indicate that the intramolecular activation of pepsinogen is inhibited by pepstatin and 'competitively' inhibited by a protein substrate (Marciniszyn *et al*, *J. biol. Chem.* **251**, 7095; 1976). This suggests that pepsin's active centre is involved in the intramolecular activation of pepsinogen. The manner by which this is accomplished in the zymogen molecule is unclear.

The results of the crystallographic and sequence studies undoubtedly will serve as models in the study of a large group of acid proteases in various biological systems. These include chymosin, the lysosomal cathepsin D and E, renin, acid proteases in blood and seminal plasma, and others It is now clear that the microbial and gastric acid proteases are derived by divergent evolution from a common ancestral gene. Cathepsin D and renin, which are similar in size to pepsin, may fall into the same evolutionary pattern. If so, then the reason that renin is derived from an acid protease in evolution might be to take advantage of the extended binding cleft. The stringent specificity requirement of eight residues in the substrate of renin thus can be accommodated These structural developments mark the beginning of more fruitful investigation into structure–function relations of the acid proteases. □

Eukaryotic chromosome structure

by Eleanor Lawrence

A Royal Society meeting on the Structure of Eukaryotic Chromosomes and Chromatin organised by Professor H. G. Callan and Dr A. Klug, was held in London on 16 and 17 February, 1977.

THE meeting covered various aspects of chromosome structure and function ranging from the fine structure of chromatin to the morphology of transcriptional units to detailed genetic studies at the molecular level.

At the level of chromatin structure, now that the string of beads model is generally accepted, attention is focused on the detailed structure of the nucleosome on the one hand and chromatin superstructure on the other. The role of 'histone' H1 in stabilising chromatin superstructure was brought out by several speakers. Although it does not seem necessary for maintaining the integrity of the nucleosome itself it seems to be essential for maintaining internucleosome connections.

P. Chambon (University of Strasbourg) described experiments which show that although all four histones H3, H4, H2A and H2B are required to assemble a complete nucleosome, H3 and H4 alone with SV40 DNA will produce small beaded structures of 75±10 Å and introduce the characteristic superhelical turns into the DNA.

The physical requirements of DNA replication and transcription have led to the proposal that the nucleosome should perhaps be able to split apart in some way, allowing free movement and replication of the DNA without completely shedding the histone. This feature has been built into current models (see for example Weintraub *et al. Cell* **9**, 409; 1976) and Chambon reported that at very low ionic strength, 'half-nucleosomes' of 90 Å diameter can be seen in the electron microscope.

At low ionic strengths A. J. Varshavsky (Institute of Molecular Biology) has obtained three types of nucleosomal particle — MN1 which appears to be the 'core' particle, MN2 which contains 170 bp DNA and has one H1 molecule associated with each particle and MN3, 200 bp and two H1 molecules per particle. He has also found an array of subnucleosomal particles ranging from 20 bp DNA lengths with no histones attached to particles containing 120 bp and all four histones, but as yet it is not possible to interpret these various subnucleosomal particles in terms of nucleosomal structure.

Biochemical studies on chromatin were reported by H. G. Zachau (University of Munich) who finds that at high salt conditions, or in the presence of divalent cations (Mg^{2+}, Ca^{2+}) DNase II produces 100 bp repeats from chromatin rather than the normal 200 bp repeats This is interpreted as a cleavage site's becoming available within the nucleosome in certain conditions. The integrity of the nucleosome is not destroyed even though the DNA is cut. Calf thymus chromatin depleted of H1 however, showed no 100 bp repeats which only reappeared when H1 was readded and Ca^{2+} was present, showing that a certain amount of superstructure is necessary for generation of the 100 bp fragments.

The structure of native chromosomes is even more difficult to probe however. A. Worcel (Princeton University) reported the finding of supercoiled loops of DNA of around 400 nucleosomes per loop in genomes of interphase nuclei of cultured *Drosophila* cells isolated in 0.9 M HCl which removes H1. In the electron microscope a very gently prepared whole genome shows a nucleofilament of 100 Å thick with no signs of a beaded structure. Worcel identifies this nucleofilament as the supercoiled thread. DNase I treatment of this preparation appears to partly release the supercoils and produce the beaded string appearance. Worcel suggested that the removal of H1 *in vivo* might be involved in the formation of transcriptionally active chromosome puffs.

R. L. Kornberg (Havard Medical School) produced evidence that nucleosome formation is random with respect to DNA sequence. If the 140 bp core DNA fragments produced from different copies of the same stretch of chromatin are always the same, single-stranded copies of opposite halves of the set of 140 bp fragments produced by digestion with exonuclease III (which digests DNA from the 3′ ends only) should not reassociate. If they carry certain sequences in common, as predicted if nucleosome formation is random with respect to DNA sequence there will be reassociation, and for single-copy DNA sequences from rat liver chromatin, Kornberg's evidence seems to show the latter to be the case He has also found variable internucleosome lengths in chromatin from the same cell type, results consonant with the finding that nucleosome formation is random.

The use of recombinant DNA techniques has revolutionised the study of gene organisation and sequence. By cloning fragments of the *Drosophila* genome and then mapping the cloned fragments back on to the polytene chromosomes D. Hogness (Stanford University) has identified various families of repeated sequences, some, like the histone genes, tandemly repeated, others, as yet functionally unidentified, dispersed throughout the genome. The histone gene repeat unit contains the five histone genes but unlike those in the sea urchin (reported by M. Birnstiel, University of Zurich), H1, H3 and H2A are transcribed off one strand and H4 and H2B off the other. A repeated sequence is found between H3 and H4 and between H2A and H2B and may possibly contain a bidirectional promoter. Tandemly repeated genes appear to be commonly regulated—all on or all off. Dispersed repeated genes on the other hand are apparently regulated independently and different ones may be turned on in different cell types or at different stages in differentiation. Sequencing of two of the dispersed families shows them to have repeated sequences of 500 bp at either end.

M. Birnstiel described continuing sequence studies on the histone gene repeat unit isolated from the sea urchin *Psammechinus miliaris*. About 25% of the total repeat unit comprising the five histone genes separated by spacer DNA has now been sequenced. The structural gene sequences agree well with those predicted on the basis of amino acid sequence, with minor differences attributable to the source of histone. Parts of the spacers which have been sequenced show no repeated sequences The *Hin*dIII spacer at the end of the unit is full of stop codons so is presumably non-coding. Some (presumably non-functional) initiation sequences have also been identified in this region. Spacer regions close to the 5′ ends of H1 and H2A show some very non-random conserved base pairs just in front of the Met initiation site. These are presumably ribosome-binding sites but this is not yet certain since they do not resemble the ribosome-binding sites identified on brome mosaic virus.

The next step would be to delete various regions of the unit and see how this affects function. A possible assay system might be that described by John Gurdon (MRC Laboratory of Molecular Biology, Cambridge) in which DNA fragments injected into the nuclei of *Xenopus* oocytes have been shown to be accurately transcribed and in some cases translated.

Transcription of the human histone genes is also being studied in Birnstiel's laboratory. The human repeat unit is found in a stretch of 14 5 kb which if transcribed together would give a polycistronic message of 50S. Such a message has been found in HeLa cells and appears to be processed to a 9S message in the cytoplasm via 17S and 28S molecules. Control of histone gene expression also appears to be post-transcriptional since G2 phase cells (when no new histones appear) still transcribe messenger.

Another approach to the study of control of gene expression is to look at the organisation of the actively transcribing regions on spread chromatin. C. D Laird (University of Washington, Seattle) described studies defining transcriptional units on *Drosophila* chromosomes. Analysis of patterns of ribonucleoprotein (RND) fibre lengths along sparsely-fibred regions of actively transcribing non-ribosomal chromatin suggest specific initiation and termination sites for each transcriptional unit. Discontinuous patterns of RNP fibre length along the transcriptional unit have been seen suggesting perhaps that the RNP fibres have been partially cleaved at some point before transcription is terminated. Contiguous transcription units have been found in embryonic *Drosophila* cells

The problem of whether the characteristic beaded structure for chromatin is maintained in actively transcribing regions is still not completely resolved, but the weight of evidence now tends to the view that it is not (W. W. Francke, German Cancer Research Centre, Heidelberg; Laird). □

Reactions with excited alpha particles: ^{12}C (α, α*)

from P. E. Hodgson

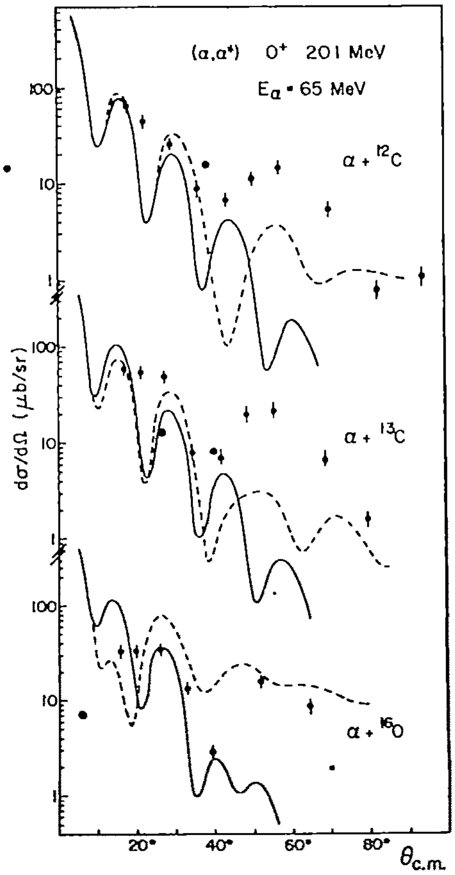
THERE is now a growing interest in using nuclear reactions with unstable emitted particles in studies of nuclear structure. Among the possible reactions, those leading to the emission of ^{8}Be are the easiest to use, because the ^{8}Be decays rapidly into two alpha particles that can be detected in coincidence. Another practicable reaction of this type is the (α, ^{2}He) reaction, where the unstable ^{2}He nucleus is detected by the coincidence of the protons into which it decays (*Nature* **264**, 511; 1976). Such reactions open up new possibilities for nuclear spectroscopy, so it is useful to see whether there are any more unstable particles that can be detected in a similar way.

One of the possibilities that has been recently realised is the inelastic scattering of alpha particles with the emission in the excited state at 20.1 MeV. This is an unbound monopole 0^+ state with a width of 0.27 MeV. An alpha particle in this state readily decays into a proton and a triton, and since the energy release is only 0.3 MeV the decay products are emitted in almost the same direction and so can easily be detected by coincidence techniques.

This reaction has recently been observed by Jahn and colleagues (*Phys. Rev. Lett.* **65B**, 339; 1976). They irradiated targets of ^{12}C, ^{13}C and ^{16}O with 65 MeV alpha particles and looked for protons and tritons emitted in coincidence. They found definite evidence that alpha particles are sometimes emitted in the state of monopole excitation, and in the case of the reaction on ^{12}C their energies corresponded to the reactions that leave the residual ^{12}C nucleus in the ground state, in the 2^+ state at 4.44 MeV and in the 3^- state at 9.64 MeV. The cross sections for these reactions are about one-thousandth of those of the corresponding reactions with the emission of alpha particles in their ground state.

Distorted wave calculations were made to see if the observed cross sections could be accounted for by existing theories. The transition matrix elements were calculated microscopically using the particle–hole model and also the collective model. The distorted waves were generated by optical potentials fitted to the elastic scattering of 65 MeV alpha particles.

These calculations agree quite well with the absolute values of the experi-



Differential cross sections for the (α, α*) reaction to the ground states of ^{12}C, ^{13}C and ^{16}O compared with distorted wave calculations using transition densities obtained from the particle–hole (solid curves) and collective (dashed curves) models.

mental cross sections, but not very well with the details of the angular distributions, as shown in the figure. This discrepancy may indicate the importance of higher order effects, in particular the two-step reactions that proceed through an intermediate state.

The detection of this reaction opens up a new area of nuclear reaction and nuclear structure studies, and in particular suggests that it may be useful to look for the (^{3}He, α^*) and (d, α^*) reactions as well. ☐

Tertiary plate motions

from Peter J. Smith

THE relative angular velocities of the Earth's lithospheric plates are now quite well known. The 'absolute' angular velocities of the plates (that is their velocities relative to the mesosphere) are known much less precisely; but if one accepts, with Solomon *et al.* (*Geophys. J.* **42**, 766; 1975), that absolute linear velocities can now be specified to within 1–2 cm yr^{-1}, then it is possible to make three statements, or groups of statements, about their relationships. First, there is a good, but not perfect, general correlation between the velocity of a plate and the fraction of the plate occupied by continent; the greater the proportion of continent the lower is the velocity. At the extremes, predominantly oceanic plates have velocities in the range 7–10 cm yr^{-1} whereas predominantly continental plates generally move at 0–2 cm yr^{-1}. Second, plates being subducted along a major proportion of their boundaries all have high velocities. And third, lithosphere in the equatorial half of the Earth has a higher r.m.s. velocity that that in the two polar quarters.

These three statements give rise, in turn, to three questions. Do continental plates move more slowly because there is a greater viscous drag at the base of the continental lithosphere than beneath the oceanic lithosphere? Do subducting plates have high velocities because of a major force exerted by the downgoing lithospheric slabs? And does the polar–equatorial velocity differential arise because of a connection between plate motions and the Earth's rotation? It is tempting to allow circumstantial evidence to answer all three questions in the affirmative, or at the very least to argue that the relationships indicated by the three statements have underlying physical causes. But equally, such relationships could well be coincidental. How, then, can the issue be decided?

The ideal way, as Solomon *et al.* (*J. geophys. Res.* **82**, 203; 1977) point out, would be "to observe a number of global plate systems, each with very different plate geometries but obeying similar physical laws". But that is obviously not possible; so Solomon and his colleagues have done the next best thing in studying the behaviour of the Earth's plates as they were arranged 55 Myr ago. Using the plate boundary map for that period already constructed by Jurdy and Van der Voo (*J. geophys. Res.* **79**, 2945; 1974) together with published values for finite plate rotations, they first determined the relative plate velocities. Absolute velocities were then calculated, assuming no net torque to be exerted on the lithosphere for a series of models having driving forces symmetrical about the plate boundaries and a plate-retarding viscous drag at the base of the lithosphere.

Needless to say, such determinations are more complicated that they are made to sound here and involve numerous assumptions, approximations and simplifications that may later give critics cause to make trouble Taken at face value, however, the results suggest that it may be dangerous to invoke particular physical processes purely on the basis of the behaviour of the plates as they are arranged now. For one thing, with the plates as they were 55 Myr ago there is apparently no correlation between r.m.s. absolute linear velocity and the fraction of the plate comprising continent, assuming the viscous drag to be uniform at the base of both the continental and oceanic lithospheres. Nor is there a clear division between fast-moving predominantly oceanic plates and slow-moving predominantly continental plates. For example, 55 Myr ago the Eurasian plate, 93% of which is continent, had a velocity about equal to that of the Pacific plate. It is true that the Eurasian plate is much slowed down if the uniform drag model is replaced by one in which there is drag only beneath the continental lithosphere; but not only would the latter model violate palaeomagnetic and other constraints, it would actually produce an increase in most continental velocities.

Fifty-five million years ago, plates with major subduction zones were not all moving rapidly either. The Farallon plate, for example, with a large fraction of its boundary under active subduction, was moving at less than half of the rate of the fastest (Kula) plate. On the other hand, the conclusion to be drawn from the polar–equatorial velocity differential is more ambiguous. The differential undoubtedly existed, albeit in a much weaker form than at present. But whereas the coordinate pole giving the maximum equatorial r.m.s. velocity is now almost coincident with the geographic pole (apparently strengthening the supposition that plate motions and the Earth's rotation are linked), it is 60° from the geographic pole for the older plate distribution. Solomon and his colleagues therefore conclude that the case for a relationship between the Earth's rotation and plate velocities is "not particularly compelling" at 55 Myr and thus, by implication, at present.

On balance, then, this new evidence suggests that the three original questions should be answered negatively; and the fact that affirmative answers are consistent with current plate motions would appear to be a coincidence. But that is not all, for Solomon *et al.* have also been able to bring their analysis to bear on the controversy over whether or not the supposed mantle plumes from which hot spots are derived are fixed in the mantle. By comparing their newly-determined absolute plate motions with observed hot spot traces, they find the fits between azimuths to be variable. For hot spot traces within the Indian and African plates the fits are within expected errors, but there are serious misfits for Pacific plate hot spots and the Icelandic hot spot. In short, not all mantle plumes appear to be fixed with respect to each other and thus not all can be fixed with respect to the mantle. ☐

A hundred years ago

HIS Majesty, the Emperor of Brazil, observed the eclipse of the moon on the evening of the 27th, at the Arcetri Observatory. The Emperor took a very lively interest in the phenomenon and discussed with acuteness the hypothesis with which Prof. Tempel, the astronomer, and Prof. Echert tried to explain the varying shades and colours in which the moon appeared during the different phases of obscuration. On Monday last his Majesty assisted at a meeting of the Anthropological Society, when Prof. Mantegazza made some interesting remarks on several Maori skulls, and Prof. Giglioli read an elaborate paper on the ethnology of Brazil.

From *Nature* **16**, March 8, 416; 1877.

review article

Binary X-ray pulsars

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There are nine known X-ray pulsars, which are widely believed to be rotating magnetised neutron stars that are accreting matter from their binary stellar companions. The observed pulse profiles provide information on the physics of the accretion process. We present representative profiles of all nine sources for comparison of general features, and we discuss some of these features in terms of the accreting neutron-star model.

THERE are about 150 known galactic X-ray sources. The most widely accepted model, for those sources not associated with supernova remnants, involves a compact object (white dwarf, neutron star, or black hole) in orbit about a relatively normal stellar companion[1]. The energy source for X-ray emission by the compact object is derived from the accretion of matter that is supplied by either a stellar wind or critical-lobe overflow from the companion[2-5]. During the first systematic X-ray sky survey with the Uhuru satellite[6], two of these sources were discovered to be regularly pulsing: Centaurus X-3 (ref. 7) (pulse period $P = 4.8$ s) and Hercules X-1 (ref. 8) ($P = 1.2$ s). Evidence for slow pulsation ($P \simeq 2$ min) in another source, GX1+4, had previously been obtained from a balloon observation[33]. For the X-ray pulsars, it seems very probable that the accreting compact objects are rotating magnetised neutron stars (see, for example, ref. 9 and references therein).

The Uhuru satellite was most sensitive to X-ray pulsations in the period range 0.2–10 s. During the past two years at least six other X-ray pulsars have been discovered. The study of X-ray pulsars has been greatly advanced by the Copernicus, Ariel V, and SAS-3 satellites. Table 1 lists the confirmed X-ray pulsars and, where known, the optical companion. The Crab Nebula pulsar, NP0532, is not included because it is evidently not in a binary system and because its pulsar mechanism is distinctly different from that of the binary X-ray pulsars.

Binary X-ray pulsars as an astrophysical tool

Binary X-ray pulsars provide at least three powerful sources of astrophysical information. First, the rotating neutron star acts as an orbiting clock that enables the observer to measure Doppler effects and thereby determine the size and shape of the orbit about the companion star. This orbital information, coupled with other supplemental data, enables one to place constraints on the masses of both the neutron star and its companion star[9] and on the size of the companion (see, for example, ref. 48). These quantities are of interest in a wide range of studies from nuclear physics to stellar evolution. Second, information on secular changes in the intrinsic pulse period can help in the understanding of the accretion process near the Alfvén surface (radius ~ 3×10^8 cm (refs 2–5)) that surrounds the neutron star. It is at this surface that the dynamics of the accreting matter are first dominated by the intense magnetic field associated with the neutron star and where the spinup or spindown torque on the neutron star is effectively applied. Third, a study of the intensity profiles of the X-ray pulsations may reveal details of the final stages of accretion onto the surface of the neutron star (radius ~ 10^6 cm). The pulse profiles may also be a direct measure of the transmission of the radiation, produced near the surface of the neutron star, through the infalling plasma. Here we discuss recent progress in the study of the pulse profiles of X-ray pulsars.

Pulse profiles

Sample X-ray pulse profiles for the nine binary X-ray pulsars listed in Table 1 are shown in Fig. 1. In all cases, the pulse profiles were obtained by integrating over ~ 10^2 to ~ 10^4 individual pulses. For several of the pulsars, the pulse profiles do not change significantly with energy over the range 2–10 keV. For others (for example, A0535+26 (ref. 29) and 3U0900−40 (ref. 39) the dependence on energy is so strong that we display data for one arbitrary narrow energy range only. It now appears that for many of the pulsars, the basic pulse profiles and their energy dependence are preserved over a time scale of at least a year. (The highly variable pulse profiles of GX1+4 seem to be an exception (refs 35 and 67). On the other hand, certain details, such as a strong secondary peak in Her X-1 (Fig. 1), are seen only on occasion.

The profiles in Fig. 1 display a wide range of complexity, ranging from single smooth intensity maxima to five maxima with additional substructure per pulse period. What general features do these profiles exhibit? (1) The profiles are all characterised by large 'duty cycles' that are in the range ~ 30–100%. This is an order of magnitude larger than those of the narrow pulses observed for radio pulsars[49]. (2) The modulation factors [defined as (maximum intensity minus minimum intensity)/maximum intensity] all fall in the range ~ 0.5–0.9. (3) At least three of the profiles exhibit sharp features. (We define such a feature as a substantial change in intensity in less than ~ 5% of a pulse period.) (4) The degree of symmetry of the pulse profiles varies widely from the nearly symmetric shapes of GX301−2 and A1118−61, which contain two times of mirror symmetry per pulse, to the asymmetric shapes of A0535+26 and 3U0900−40 that contain no symmetry times. (5) There is no significant trend in the complexity of the profiles as a function of pulse period. For example, the pulse profiles of SMC X-1 and GX301−2 are somewhat similar in form, as are those of Her X-1 and 3U0900+40. (6) There is a tendency for the profiles of some of the slow pulsars (for example, A0535+26 and 3U0900−40) to vary greatly with energy; at higher energies (not shown in Fig. 1), these pulse profiles become simpler than in the 1–10-keV range.

We note, as have other workers, the apparent existence of a gap in the period distribution of X-ray pulsars, ranging from P ~ 5 s to P ~ 100 s. As of this writing, the gap seems to be genuine within the obvious statistical limits and is probably not an observational effect.

Pulsation mechanisms

The most widely accepted mechanism for producing pulsed X-ray emission invokes the chanelling of accreted matter to the surface of a rotating neutron star by intense magnetic fields (~ 10^{12}–10^{13} G (refs 2–5)). For all of the X-ray pulsars, the pulse profiles are probably determined by the following parameters: the mass accretion rate; the magnetic field configuration near the surface (that is, dipole field or fields of higher complexity); the relative angles between the rotation axis, magnetic axis, accretion disk (if any), and the observer; and the rotation period. Aside from obviously determining the pulse period, the rotation period probably influences the manner in which the accretion across the Alfvén surface occurs[50].

These considerations suggest two explanations for the production of X-ray pulsations. The first requires a temporal modulation of the accretion of matter onto the surface of the neutron star (ref. 51 and M. Rees, personal communication). This might occur if the accreting material originates in a stellar wind from the companion star, passes through a shock front near the neutron star, and accretes without the formation of an accretion disk (see, for example, S. L. Shapiro and A. P. Lightman, preprint). The accretion may then take place at a variable rate that depends upon the instantaneous angle between the magnetic axis and the direction of the stellar wind. A temporal modulation of accreted material could also occur if an accretion disk surrounds the neutron star and the rotation axis of the neutron star is not perpendicular to the disk. A non-aligned magnetic axis would then cause the accretion disk to pass through different magnetic latitudes as a function of time[51]. In either of these cases the pulse profiles would represent, at least in part, the modulation of the rate of matter reaching the surface. The former case, however, predicts an orbital-phase dependence for the pulse profiles (M. Rees, personal communication) that has not been observed to date (unpublished SAS-3 data). In the latter case, the explanation probably fails at least for Her X-1 (which is widely believed to accrete from a disk), where pulse structure has been observed on a time scale of ~ 20 ms (Fig. 1). Since the free-fall time from the Alfvén surface is ~ 0.2 s, it is doubtful that significantly smaller time structure can result from temporal modulation of the accretion rate.

Table 1 Binary X-ray pulsars

Source	Pulse period (s)	References			Optical companion
		Discovery of pulsing	Data in Fig. 1	Other observations of pulsation	
SMC X-1	0.715	10	10	11	Sk 160 (refs 12, 13)
Her X-1	1.24	8	14	15–21, 66	HZ Her (ref. 22)
Cen X-3	4.84	7	23	24–26 and F. Li, unpublished SAS-3 data.	Krzeminski's star[27]
A0535 + 26*	104	28	29	30,31	HDE 245770? (ref. 32)
GX1 + 4	122	33†	67	35	M giant star?[36,37]
3U0900−40	283	38	39		HD 77581 (ref. 40)
A1118−61*	405	41	41		RS Cen? (ref. 42) Be star? (ref. 43)
GX301−2	696	34	68	J. H. Swank *et al.*, preprint	WRA 977 (refs 44, 45)
3U0352 + 30	835	46	J. G. Jernigan, unpublished SAS-3 data		X Per (ref. 47)

* Transient source.

† Confirmation of the presence of pulsation was obtained by White *et al* [34].

An alternative and more likely picture[3–5,52–57] for producing the pulsations requires the funnelling of matter along the magnetic field lines to the surface of the neutron star, where the released gravitational potential energy heats a small area on the surface (~ 1 km^2) to extremely high temperatures ($\gtrsim 10^8$ K). The radiation from each of the magnetic polar caps distribution that reflects both the location of the surface hotspots (there may be more than two) might be approximately isotropic or it might be beamed by a cyclotron emission process[52]. In either case, the emergent radiation must then pass through and interact with the infalling magnetised plasma (see, for example, refs 52, 55 and 56). Near the bases of the accretion columns, the magnetic field strengths and particle densities may well be as high as ~ 10^{13} G and ~ 10^{22} cm^{-3}, respectively. A variety of photon absorption, emission and scattering processes are possible in these extreme conditions (for example, free-free absorption[58,59], cyclotron processes[50,52,60], and anisotropic Thomson scattering[58,61]), all influenced by the strong magnetic field. With the wide ranges of temperature, density, and magnetic field strength that probably occur within the accreting matter, the radiative transfer problem is exceedingly complicated and has heretofore been quantitatively investigated only for specific scattering mechanisms and in various limiting cases of temperature and magnetic field[52,55,56,62].

After the radiation has diffused through the infalling medium, it should emerge as X radiation and will have an angular distribution that reflects both the location of the surface hotspots and the radiative transfer through the medium (including possible transfer through matter near the Alfvén surface[63,64]). This distribution may, in the first approximation and for the case of a simple dipole field, be a function of magnetic latitude only. For a more complex magnetic-field configuration, the emergent X-ray distribution can depend on magnetic longitude as well as latitude. As the neutron star rotates about its axis (which is presumably not aligned with the magnetic axis), a distant observer will pass over a range of magnetic coordinates and will thereby observe a corresponding X-ray pulse structure. It is immediately evident (Fig. 1) that the complex pulse profiles observed for several sources (for example Her X-1, A0535+26, and 3U0900−40) cannot be only the result of scattering and absorption in accretion columns associated with a simpel dipole field, which would yield profiles with two times of mirror symmetry per pulse.

Conclusions

Of approximately 150 galactic X-ray sources, nine are known to be binary X-ray pulsars. The list is not likely to expand quickly to include many of the remaining sources. Numerous galactic sources have been searched for regular pulsation over a wide range of possible pulse periods with negative results (limits on pulsed fraction of a few percent; unpublished SAS-3 data of S. Rappaport). However, a significant amount of information on the nature of binary X-ray pulsars has already been developed from studies of the nine pulsars discussed in this article. Further observations will probably reveal some new pulsars and these will improve the quality of the statistical information on the properties of these sources. For example, does a genuine gap exist in the pulse period distribution? Are there any systematic trends in the typical pulse profiles as a function of pulse period?

It is possible that the futher study of binary X-ray pulsars will not lead in the forseeable future to any conclusive understanding of the relevant physics and astrophysics involved. This situation may have already emerged in the study of radio pulsars[46]. It might be argued that because binary X-ray pulsars are not isolated objects, their physics may be even more difficult to unravel than that of radio pulsars. It is our belief, however, that while the picture is indeed complex, we already have a sound qualitative idea of the underlying physical processes at work in these systems, and the prospects for further significant advances in our quantitative understanding are therefore encouraging.

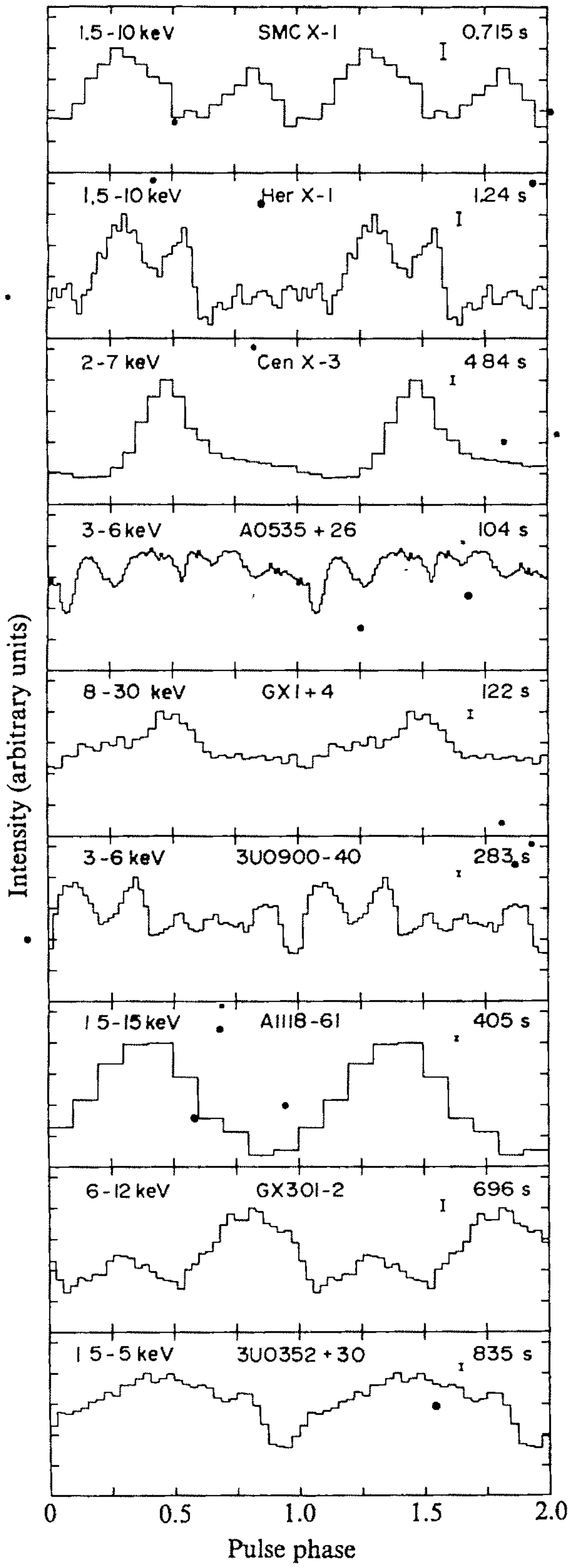

Fig. 1 Pulse profiles for the nine X-ray pulsars that are thought to be in binary systems. In each case, the data (Table 1) are folded modulo the pulse period and plotted against pulse phase for two complete cycles. The approximate pulse periods and energy intervals are indicated for each pulsar. Non-source background counting rates have been subtracted. A typical ±1σ error bar, derived from photon counting statistics, is indicated for each pulse profile.

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articles

Distances of the Virgo, Fornax and Hydra clusters of galaxies and the local value of the Hubble ratio

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Distances of three nearby clusters of galaxies derived from recent observations of the brightest globular clusters in the brightest galaxies are now in good agreement with other distance indicators and suggest a local value of the Hubble 'constant' $H = 86 \pm 9$ *km* s^{-1} *Mpc*$^{-1}$.

RECENT observations have renewed interest in the concept of the brightest globular star clusters in galaxies as stochastic distance indicators subject to size-of-sample effects[1,2]. The thorough study by Hanes[3] of the apparent magnitudes of the brightest globular clusters in 12 of the brightest galaxies in the Virgo I cluster facilitates a better determination of the cluster population and luminosity function in relation to the luminosity of the parent galaxy. Smith and Weedman[4] reported very faint objects, probably globular clusters, at magnitudes $B \simeq 23$ to 24 and fainter, around NGC 3311, the brightest galaxy in the Hydra I cluster. Dawe and Dickens[5] described faint objects, possibly globular clusters, appearing at magnitudes $B \geqslant 21.0 \pm 0.6$ (m.e.) around several of the brighter galaxies in the Fornax I cluster.

In this paper I first revise the basic parameters, $\bar{M}$(CL) and $\sigma(M)$, the mean and dispersion of the gaussian model used to estimate the expectation value $M_B(1)$ of the brightest globular cluster as a function of the absolute magnitude $M_B{}^*(G)$ of the parent galaxy. Second, I introduce a new version of the brightest cluster method which does not require successive approximations. Third, I derive the absolute distance modulus μ_0 of the Virgo I cluster from $M_B(1)$ in the brightest elliptical and lenticular members. Fourth, I derive the distance modulus difference $\delta\mu_0$ between For I and Vir I from several distance indicators, including the suspected globular clusters. Fifth, I derive an approximate modulus for Hya I from the apparent magnitudes and diameters of its brightest members and from $M_B(1)$ in NGC 3311. Finally, I derive the values of the velocity–distance ratio (Hubble 'constant') in the directions of these three nearby clusters, two of which are well outside the boundaries of the Local Supercluster. The result '$H = 86 \pm 9$ km s^{-1} Mpc^{-1}' is in general agreement with several independent estimates[6–9], but in disagreement with others[10,11].

Luminosity function of globular clusters and expectation value of $M_B(1)$

The luminosity function of galactic globular clusters is very nearly gaussian (Fig. 1). The cumulative frequency function of the absolute magnitudes listed for 105 clusters by Kukarkin (ref. 12, Table F) is well approximated by a Laplace–Gauss integral with mean $\bar{M}(\text{CL}) = -7.2$ and $\sigma(M) = 1.2$ mag (dashed curve). The better-defined brighter half, which is of more immediate interest for the evaluation of $M_B(1)$, is more closely fitted by $M_V(\text{CL}) = -7.3$, $\sigma(M < \bar{M}) = 1.1$ mag (solid curve). The latter parameters are adopted in this article. The distance scale used by Kukarkin is defined by the mean magnitude of the RR Lyrae variables in 89 clusters $\bar{M}_V(\text{RR}) = +0.86$

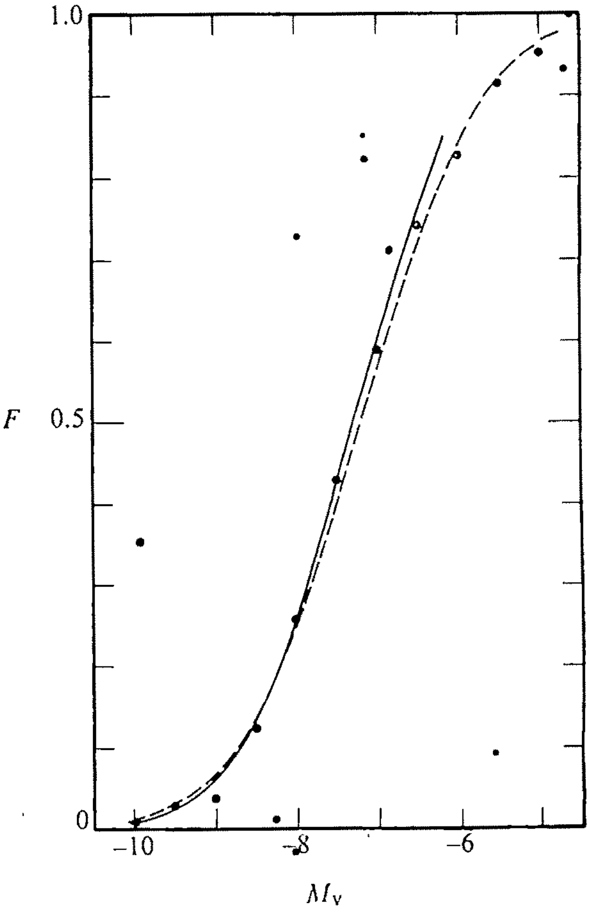

Fig. 1 Cumulative luminosity function $F = N(\text{CL})M_V)/N_T$ of 105 galactic globular clusters from Kukarkin's catalogue. The dashed curve is a Laplace–Gauss integral with mean $\bar{M}_V(\text{CL}) = -7.2$ and dispersion $\sigma(M) = 1.2$ mag; the solid curve with $\bar{M}_V(\text{CL}) \doteq -7.3$, $\sigma(M) = 1.1$ mag gives a better fit of the brighter half of the luminosity function.

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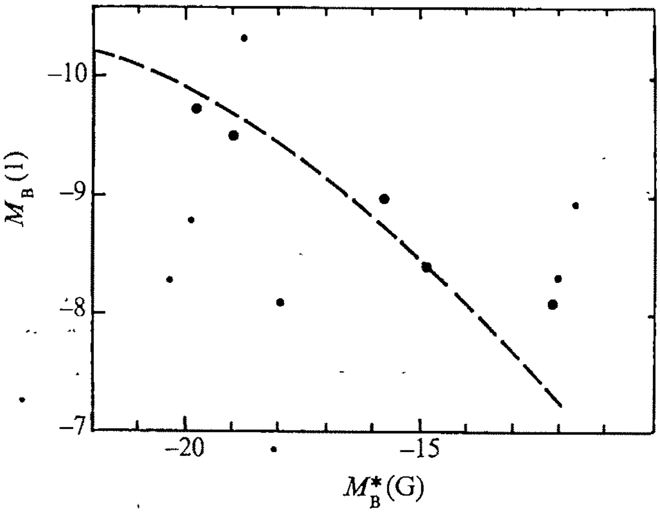

Fig. 2 Observed and computed absolute magnitude $M_B(1)$ of brightest globular cluster as a function of absolute magnitude of spheroidal component of galaxy $M_B^*(G)$. Dashed curve is from statistical theory.

(range 0.57–1.33) (ref. 12 Table 8). A similar fit of the B-band luminosity function gives $\overline{M}_B = -6.55$ and $\sigma(\overline{M}_B < M_B) = 1.1 \pm 0.05$.

The derived expectation value $M_B(1)$ of the brightest cluster depicted in Fig. 2 as a function of $M_B^*(G)$ depends also on the relation between the total cluster population N and $M_B^*(G)$. The adopted relation, $\log N = -0.3\ (M_B^*(G) + 11.0)$, is a corrected average of those used in refs 1 and 2. It is rather uncertain and further studies will be necessary to establish this relation more firmly and to determine whether N depends also on other factors. For the present the main justification of the adopted relation is its agreement with empirical data on B_1(CL) for five galaxies of the Local Group covering at least a 5-magnitude range in $M_B^*(G)$ (Table 1). Except for the Fornax dwarf where statistical fluctuations may be expected because of the very small sample ($N = 5$), the O–C residuals are all within ± 0.25 mag which is well within the errors of the data and approximations of the model.

$M_B(1) - M_B^*(G) = B_1(\mathrm{CL}) - B_T^*(G)$ as distance indicator

Derivation of the distance modulus from Fig. 2 requires in general several successive (but rapidly converging) approximations as explained in ref. 1. The need for successive approximations is eliminated in a variant of the method using the difference of apparent magnitudes between the brightest cluster B_1(CL) and the galaxy $B_T^*(G)$ to calculate directly the absolute magnitude $M_B^*(G)$ and apparent distance modulus $\mu = B_T^*(G) - M_B^*(G)$ of the galaxy. Figure 3 shows the relationship between $M_B^*(G)$ and $M_B(1) - M_B^*(G) = B_1(\mathrm{CL}) - B_T^*(G)$ derived from the statistical model and the data in Table 1.

A convenient linear approximation of the relationship for non-dwarf galaxies ($M_B^* \leqslant -16.0$) is

$$M_B^*(G) = -16.0 - 1.20\ (\Delta m_1 - 7) \qquad (1)$$

where $\Delta m_1 = B_1(\mathrm{CL}) - B_T^*(G) \geqslant 7$. It is because $\delta M_B(1)/\delta M_B^*(G) = 0.2$ only that $M_B(1)$ was initially believed to be a constant[13,14]. Equation (1) can be regarded as an empirical fit of the data which applies irrespective of the validity or otherwise of the underlying statistical model.

Distance modulus of the Vir I cluster

Application of equation (1) to the six brightest E galaxies in the cluster, including M87, with $\langle B_T^*(G) \rangle = 9.9$ and $\langle B(\mathrm{CL}) \rangle = 20.3$, that is, $\Delta m_1 = 10.4$, leads to $M_B^*(G) = -20.1$, and $\mu_B = 30.0$ (the brightest cluster magnitude B_1(CL) is defined statistically by a gaussian fit of the luminosity function[16]; it does not depend on the highly uncertain identification of any particularly cluster as the brightest. Galaxy magnitudes $B_T^*(G)$ are taken from ref. 15). The five fainter E and L galaxies in the Hanes[3] study have $\langle B_T^*(G) \rangle = 10.9$ and $\langle B_1(\mathrm{CL}) \rangle = 20.8$, then $\Delta m_1 = 9.9$, $M_B^*(G) = -19.5$, and $\mu_B = 30.4$. This value is preferred because it does not require an extrapolation of equation (1) beyond the range of $M_B^*(G)$ in Table 1. The mean error of the distance modulus is estimated at 0.3 mag, arising from the following components: 0.1 on B_1(CL), 0.1 on $\overline{M}_B$(CL), 0.15 on the median magnitude of cluster variables, 0.15 on the basic data[1], and 0.15 from the statistical model and related approximations, including neglect of cluster depth. The adopted modulus of Vir I from the brightest globular clusters is, then, $\mu = 30.4 \pm 0.3$; it is compared in Table 2 with estimates by other methods. The disagreement between the brightest cluster method and other methods[6] has been greatly

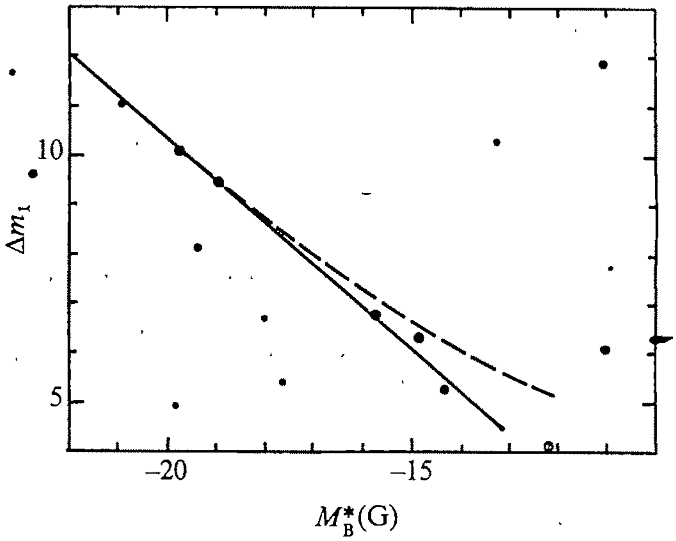

Fig. 3 Relation between magnitude difference $\Delta M_1 = B_1(\mathrm{CL}) - B_T^*(G)$ and absolute magnitude of spheroidal component of galaxy $M_B^*(G)$. The dashed curve is from theory ($\sigma = 1.1$ mag) and the linear approximation is from equation (1).

Table 1 Observed and calculated absolute magnitudes of brightest globular clusters

Galaxy	$M_B^*(G)$	$M_B(1)$ (Observed)	(O−C) $\sigma = 1.1$	Δm_1 CL−G	$M_B^*(G)$ (from equation (1))
M31*	−19.8	−9.75	+0.2	10.05	-19.6_5
Galaxy*	−19.0:	−9.5	+0.2	9.5:	−19.0:
N205	−15.8	−9.0	−0.25	6.8	-15.7_5
N185†	−14.9:	−8.4	0.0	6.4:	(−15.3:)
Fornax	−12.2	−8.1	−0.7:	4.1	(−12.5:)

*$M_B^*(G)$ for spheroidal component only[1,6,21].

†$B_T(G) = 10.1$ (ref. 15), $\mu = 24.9$, and $B_{1E} = 16.5 \pm 0.5$ from uncertain $V_1 = 16.2 \pm 0.45$ (ref. 22) and $B_{2,3} = 17.45 \pm 0.05$ (ref. 22) corrected for average $\Delta m(2\text{–}1) = 1.15 \pm 0.25$ between second and first ranked clusters in N205 and Fornax.

Table 2 Apparent distance modulus of the Virgo I cluster

Source	Method	μ±m.e.
Holmberg[23]	Corrected colour–surface brightness of spirals	30.76
Heidmann *et al.*[7] (Table XXVIII)	Diameter–luminosity relation for spirals in S-cloud	(30.9 ±0.5:)
de Vaucouleurs[24] (Table 2, 1965 data)	Group distances from $B(0)$ and $D(0)$, corrected for size of sample	(30.5)
de Vaucouleurs[24]	Group distances from $B(0)$ and $D(0)$, corrected for size of sample (E cluster)	30.87±0.2:
(Table A1, 1973 data)	Group distances from $B(0)$ and $D(0)$, corrected for size of sample (S cloud)	30.51±0.2:
This article	Brightest globular clusters	30.4±0.3
	Weighted mean <μ>	30.65±0.15

Table 3 Distance modulus difference between For I and Vir I clusters

Source	Method	$\delta\mu_0$	
de Vaucouleurs[25] (Tables XXI, XXII)	Ratio of mean corrected diameters of E and L galaxies in regions VI (Pavo-Indus) and IIa (For I) divided by ratio of mean (r) rings diameters in region VI and Virgo I (0′.85/1′.25)	0.52 (0.12)	(E) (L)
de Vaucouleurs[23] (Table 2, 1965 data, and Table A1, 1973 data)	Group distances from magnitudes and diameters of five brightest and/or largest galaxies corrected for size of sample	(0.8) 0.68	 (E,L)
Dawe and Dickens[5]	M_1(CL) uncorrected for size-of-sample	(0.6)	(E)
This article	Corrected magnitudes and diameters of three brightest and/or largest galaxies adjusted for size of sample from RC 2 data[16]	0.97	(E,L)
	Weighted mean	0.7±0.15	

reduced (actually reversed) and all methods now agree within their errors with a mean modulus $\mu = 30.65 \pm 0.15$. Correcting μ for $A_B = 0.20$ mag of galactic extinction, the geometric distance modulus is $\mu_0 = 30.45 \pm 0.15$, corresponding to Δ(Vir I) = 12.3 (1±0.07) Mpc.

Distance modulus difference $\delta\mu_0$ between For I and Vir I

Several estimates of $\delta\mu_0$ are collected in Table 3; $\delta\mu \simeq \delta\mu_0$ because galactic extinction is very nearly the same in the directions of Vir I ($A_B = 0.20$ mag) and For I ($A_B = 0.21$ mag) in spite of the difference in galactic latitudes[25]. Considering the uncertainties of the basic data and underlying assumptions, the agreement between the different methods is rather good; the unweighted mean (rejecting low-weight of preliminary values in parentheses) is $\overline{\delta\mu_0} = +0.7 \pm 0.15$, implying a distance ratio Δ(For I)/Δ(Vir I) = 1.38 (1±0.07), a geometric distance modulus $\mu_0 = 31.15 \pm 0.2$ ($\mu = 31.36$), and a distance Δ (For I) = 17.0 (1±0.10) Mpc.

Table 4 Distance modulus of Hya I cluster

Method	μ_0
B_1(CL) and equation (1)	33.0
ΔB_1(CL) from M 31 ($\mu = 24.8$)	33.0
$\Delta\mu_0$ (Vir-Hya) = 2.55±0.2: from 3 brightest and largest E galaxies	33.0
Adopted mean	33.0±0.2

Approximate distance modulus of Hya I cluster

The brightest globular cluster method now agrees with all other reliable distance indicators, so an approximate value of Δ(Hya I)

Table 5 Mean corrected radial velocities of Vir I, For I and Hya I clusters

	Source	E, L Types $\overline{V}_0$±m.e.	N	All types $\overline{V}_0$±m.e.	N
a	Tammann[17]			1,141±60	122
b	G. and A. de Vaucouleurs[18]	1,012±71	42	1,054±66	97
c	Sandage and Tammann[9,19]			1,111±75	95
d	This article	973±120	21		
	Adopted Vir I	1,000±70		1,100±60	
e	de Vaucouleurs[24]			1,464±98	12
f	Sandage[10]	1,527	4		
g	This article	1,462±108	12	1,448±89	17
	Adopted For I	1,450±100		1,450±90	
h	Vidal and Peterson[25]			3,233±228	15
i	Sandage[11]			3,697	7
j	This article	3,647±105	6	3,611±200	10
	Adopted Hya I	3,650±100		3,500±200	

a, Galaxies of all types in extended Virgo area, including 'Roberts correction' for spirals. *b*, 42 Elliptical and lenticular galaxies in 12° E cluster, and 1296±71 from 26 Sa to Scd galaxies in S cloud; weighted mean is adopted for all types. *c*, 95 Galaxies of all types in 12° classical cluster (no correction) *d*, 21 E and L galaxies in 3° = 0.64 Mpc core of E cluster (12h22 to 12h34, +11°. 4 to +14°.3, 1950), mean is 981±101 (n = 19) if 2 objects with large residuals (N4406, N4473) are rejected. *e*, Galaxies of all types in For I area. *f*, 4 E and L galaxies in For I area. *g*, 12 E, L galaxies and 17 of all types in 1°.4 = 0.4 Mpc core of cluster. *h*, 15 Galaxies of all types in cluster area, includes 10 anonymous objects with $\overline{V}_0$ = 3162. *i*, 7 Galaxies of all types in cluster (listed as Hya II), includes 3 anonymous with $\overline{V}_0$ = 4280. *j*, 6 E, L galaxies and 10 of all types in 0°.5 = 0.35 Mpc core of cluster[16].

Table 6 Velocity-distance ratios for Vir I, For I and Hya I clusters

Cluster	SGL	SGB	V_0 (km s^{-1})	Δ (Mpc)	'H'
Vir I (E)	103°	−2°	1,000 (1±0.07)	12.3 (1±0.07)	81 (1±0.10)
Vir I (E+S)	103°	−2°	1,100 (1±0.06)	12.3 (1±0.07)	89 (1±0.10)
For I (E)	262°	−42°	1,450 (1±0.07)	17.0 (1±0.10)	85 (1±0.12)
For I (E+S)	262°	−42°	1,450 (1±0.06)	17.0 (1±0.10)	85 (1±0.12)
Hya I (E)	139°	−38°	3,650 (1±0.06)	40.5 (1±0.1:)	91 (1±0.12)
Hya I (E+S)	139°	−38°	3,500 (1±0.03)	40.5 (1±0.1:)	87 (1±0.1:)
			Adopted mean		86 (1±0.10)

may be derived from the work of Smith and Weedman[4]. From their Fig. 2 the apparent magnitude of the brightest clusters in NGC 3311 may be estimated provisionally at $22.8 < B_1(\text{CL}) < 23.8$, say $B_1(\text{CL}) = 23.3 \pm 0.2$ The Galaxy, one of the two brightest ellipticals in Hya I, has a total magnitude $B_T(G) = 12.7 \pm 0.1$ (ref. 15); thus $\Delta m_1 = 10.6$ and, from equation (1), $M_B{}^*(G) = -20.3$, $M_1(\text{CL}) = -9.7$ and $\mu = 33.0$. Since $M_B{}^*(G)$ for M 31 and N3311 differ little, a direct comparison of the first-ranked clusters is legitimate; then $\Delta\mu(1) = 23.3 - 15.05 = +8.25$ and $\mu_0(\text{Hya I}) = (24.8-0.43) + (8.25-0.38) = 33.0$. From a comparison of corrected apparent magnitudes and diameters of the three brightest elliptical members in each cluster, the differential modulus is $\Delta\mu_0$ (Hya–Vir) $= 2.55 \pm 0.1$: and $\mu_0(\text{Hya}) = 33.0 \pm 0.2$. The adopted mean of the values collected in Table 4 is $\mu_0 = 33.0 \pm 0.2$ and $\Delta(\text{Hya I}) = 40.5$ $(1 \pm 0.10{:})$ Mpc (with a galactic extinction $A_B = 0.38$ (ref. 15)).

Local value of the Hubble ratio

Recent or new estimates of the mean corrected redshifts of the Vir I, For I and Hya I clusters are collected in Table 5. To bypass the controversy[17–19] concerning the equality or otherwise of the mean redshifts of the Virgo I E cluster and S cloud, and because For I and Hya I are both E-type clusters with dense cores, the mean redshift of the 3° = 0.6 Mpc core of the Virgo E-cluster may be considered more appropriate for a direct comparison with the other two clusters. (Also, spirals in the S-cloud have a much larger velocity dispersion[18,19] and may degrade rather than increase the precision of $\overline{V}_0$.)

The comparison is summarised in Table 6 where the values of the mean velocity-distance ratio $'H' = \overline{V}_0/\Delta$ are derived from the adopted redshifts and distances. It is clear that the three clusters are in general agreement with much other evidence to support a mean value $'H' = 86\ (1 \pm 0.1)$ km s^{-1} Mpc^{-1} as the proper local average of the Hubble ratio, significantly higher than the often quoted values of 57 (ref. 10) to 50 (ref. 11). This persistent disagreement underlines the importance of current efforts to derive the distances of clusters more distant than Vir I in several directions well away from the centre of the Local Supercluster.

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Nile Cone: Late Quaternary stratigraphy and sediment dispersal

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The cyclic nature of Late Quaternary Nile Cone sediments is a response to major climatic oscillations, including marked changes in sea level, Nile River discharge and sediment influx and water mass stratification. Deposits have accumulated most rapidly north-west of the Nile Rosetta Branch since the Early Würm A broad area of low sedimentation on the Cone, initiated during the Holocene sea-level rise, is predicted to increase in size as a result of the Aswan High Dam and modification of Nile River flow.

THE Nile Cone, largest of the Mediterranean deep-sea fans, has a complex history related to the large-scale tectonics of the Levantine Basin[1–3] and evolution of the Nile River which has served as its major sediment source Deposition on the Cone in the Quaternary has differed from that on most other large subsea fans in one important aspect, that is, sediments carried seaward of the Nile accumulated in a relatively small enclosed water body and thus have been extremely susceptible to climatic changes affecting this region. The deposits record modifications of eustatic sea level, Nile River, headwater migration and flow, physical oceanography, including current patterns and stratification,

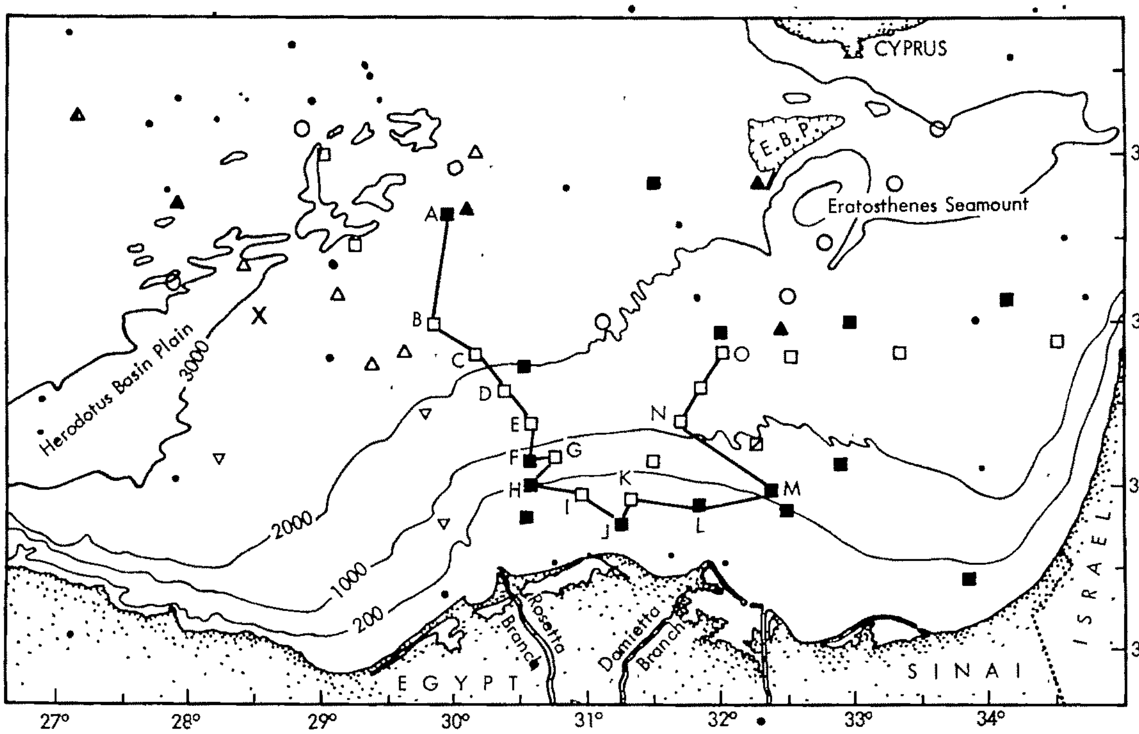

Fig. 1 Chart of the southeastern Mediterranean showing Nile Cone cores examined in this study. EBP, Eratosthenes Basin plain. Depth in metres. ○, Chain 61; ■, Pillsbury 6508; □, Pillsbury 6510; △, Vema 10; ▽, Vema 14; ▲, R. D. Conrad 9; ×, Deep Sea Drilling Project.

and biogenic productivity in front of the Nile. The emplacement of the Aswan High Dam and near-cessation of fluvial input seaward of the delta[4,5] are now altering the natural evolution of the Egyptian Shelf and Cone. The detailing of Late Pleistocene to Holocene depositional patterns, of which little is known, is of value in predicting changes likely to occur seaward of the subaerial delta.

The stratigraphy of the eastern Mediterranean is discussed in a number of studies[6–15]. Our earlier investigations emphasised the remarkable variety of sediment types forming the Cone and their cyclic nature[16,17]. Here, time and spatial variations of the uppermost sedimentary cycles are defined. We believe this cyclicity records large-scale Würm to Recent climatic events which have directly affected the depositional history of the southeastern Mediterranean.

This study entails analysis of 47 piston and gravity cores (Fig. 1), of about 1–15 m length, from the collections of: University of Miami RV Pillsbury cruise 6508 (15 gravity cores) and cruise 6510 (13 gravity and three piston cores); Woods Hole Oceanographic Institution RV Chain cruise 61 (five piston cores), Lamont-Doherty Geological Observatory RV Vema cruise 10 (five piston cores) and cruise 14 (three piston cores), and RV Conrad cruise 9 (three piston cores). Pillsbury and Chain cores are the best preserved. Core analysis includes X radiography, size analysis, total carbonate and organic matter, SEM, clay and sand fraction composition.

A total of 34 Pillsbury core samples provided radiocarbon dates (Table 1, R Stuckenrath, Smithsonian Radiocarbon Laboratory, personal communication). Dates were obtained from bulk samples about 10–30 cm in length. This method allows a small amount of core section to be used for dating and our tests show that dates so obtained are comparable to those obtained using only the sand-size planktonic foraminifera and pteropod fraction (cf. Pillsbury 6510-14 core section 21–61 cm). X-radiograph analysis reveals that sapropel samples selected for radiocarbon dating are finely laminated and not contaminated by bioturbation.

Correlation shows that sequences in the narrow diameter (4 cm) gravity Pillsbury cores are usually compressed relative to those in wider diameter (6.5-cm) piston cores, that is, 100 cm of piston core equals about 60 cm of gravity core section (cf. comparative scales along logs in Figs. 2). Piston and gravity cores of the same diameter (6.5 cm) may show a reverse trend, that is, piston core sections compressed relative to gravity core sections (C. Summerhayes, personal communication). Thickness data from piston cores serve as the basis for calculating sedimentation rates; thicknesses in gravity cores are recalculated to correlate with those of piston cores.

Late Quaternary stratigraphy

The simplified graphic logs of 15 of the 47 cores studied in detail are shown in Fig. 2; these are cores for which carbon-14 dates are available. Two well preserved piston cores, Pillsbury 6510-4 (1190 cm long) and 6510-14 (1528 cm long), collected respectively north-east of the Damietta Branch (1634-m depth; Fig. 1, core O) and north-west of the Rosetta Branch (2295 m; Fig. 1, core C) are used as types sections for the chrono- and lithostratigraphical analysis of the Late Quaternary sequence. These cores, collected in two different Cone sectors, show a complete sequence of sediment types without any apparent discontinuity.

The cores on the shelf and upper slope (to about 450-m depth) include three major sediment types in order of decreasing importance grey homogeneous mud (usually bioturbated), characterised by pelecypods and other benthonic and planktonic components; bioclastic neritic calcareous sand and gravel ('maerl') which display lithological transitions with the grey mud type; and terrigenous shelly sand and silt, including nearshore and pro-deltaic deposits. Two other sediment types, well-represented on the shelf[18] but not usually recovered in cores, are coarse, well-sorted, quartz-rich sands (reworked relict coastal sediment) and calcareous deposits of encrusting algal origin. The upper part of cores on the outer shelf and upper slope usually displays a distinct neritic calcareous sand dated between about 2700 and 2000 yr b.p. The upper part of cores on the inner shelf displays this horizon, and may also show a similar calcareous layer of sub-recent to modern age above it near the core top (c.f. core Pillsbury 6508-41, Fig. 2). In mid-shelf cores, a lower layer of neritic calcareous sand, separated from the upper calcareous sand by grey mud, is dated at about 16,000 yr b.p. (Pillsbury core 6508-42). The short length of shelf cores precludes an analysis of older Quaternary facies.

Cores collected at depths beyond 550 m on the slope consist essentially of six sediment types: turbiditic sand and silt, turbiditic mud, hemipelagic mud, calcareous ooze, organic ooze and proto-sapropel, and sapropel (defined in ref. 16). Also distinguished is a yellowish-orange to light brown mud termed oxidised layer (OL); where present, it usually caps a sapropel sequence. Clean, well-sorted quartz sands also are present in a few cores on the Cone seaward of Rosetta Branch. In both Pillsbury type cores, hemipelagic mud predominates (about 50% of the total core section). The repetition of sediment types which recur from bottom to top is noteworthy: grey hemipelagic mud→sapropel and organic ooze→calcareous ooze. Each complete cycle is defined as a cyclothem[17]. Incomplete silt-to-mud turbidite sequences occur indiscriminately throughout each cyclothem (Fig. 2).

Table 1 Radiocarbon dates based on bulk sample (except P6510-14P, 21–61 cm) from cores selected in different physiographic areas of the Nile Cone and adjacent areas (G, gravity core; P, piston core; p, pilot gravity core)

Core	Type	Depth (cm)	Sediment type	Radiocarbon date (yr b.p.)	Notations
P6508-34	G	0–8	Calcareous ooze	2,795±75	
		100–115	Organic ooze	9,305±85	
P6508-38	G	7–17	Calcareous ooze	2,320±70	
		17–30	Hemipelagic mud	3,265±70	
P6508-38	G	14–25	Biogenic sandy mud	2,705±70	
		45–60	Grey shallow water mud	—	Sample too small
P6508-41	G	24–33	Biogenic sand	2,675±70	
P6508-42	G	88–100	Biogenic muddy sand	16,100±300*	
P6508-45	G	90–110	Grey mud	10,375±290*	
P6510-4	p	25–33	Organic ooze	6,665±295*	
		74–82	Calcareous ooze	17,200±275	
		95–105	Calcareous ooze	19,100±200	
P6510-4	P	11–18	Oxidised layer	5,785±90	
		21–28	Organic ooze	6,575±160*	
		51–60	Sapropel	8,215±160*	
		101–110	Hemipelagic mud	10,475±140*	
		120–129	Hemipelagic mud	15,390±175	
		302–318	Hemipelagic mud	25,875±480	
		421–429	Calcareous ooze/hemipelagic mud	28,725±840	
		461–469	Organic ooze	>40,000	Apparent age beyond theoretical limits of detection is 45,800±1300
		502–518	Hemipelagic mud/calcareous ooze	>40,000	
		561–578	Organic ooze	>40,000*	
		633–648	Hemipelagic mud/calcareous ooze	>40,000	Apparent age beyond theoretical limits of detection is 46,800±1400
P6510-6	G	40–75	Organic ooze	6,715±200*	
		120–142	Calcareous ooze/hemipelagic mud	16,900±150	
P6510-8	G	102–113	Biogenic sandy mud	—	Sample too small
P6510-10	G	0–10	Biogenic sand	19,700±90	
		90–102	Biogenic muddy sand	—	Sample too small
P6510-11	G	11–20	Calcareous ooze	2,630±70	
P6510-12	G	10–20	Hemipelagic mud	4,350±80	
		80–95	Sapropel	—	Sample too small
P6510-13	G	110–123	Calcareous ooze	17,300±170	
P6510-14	P	21–61	Hemipelagic (turbiditic) mud	5,985±700	Fraction coarser than 63 μm
		21–61	Hemipelagic (turbiditic) mud	6,325±700	Fraction finer than 63 μm
		317–360	Hemipelagic (turbiditic) mud	22,500±290	
		531–569	Calcareous ooze/hemipelagic mud	32,600±1000	
		801–839	Sapropel–organic ooze	38,600±700*	
P6510-15	G	100–114	Calcareous ooze/hemipelagic mud	18,830±295	

*Small sample, diluted and counted at reduced pressure.

The upper three cyclothems present distinctive suites of lithological characters enabling each to be identified in the study area. The uppermost (cyclothem 1) is distinguished by an oxidised layer above a well-marked sapropel (S_1) layer 8–20 cm thick, thick organic ooze layers above and below the sapropel, and a general absence of turbiditic layers above the oxidised layer. The second (cyclothem 2) comprises a poorly developed sapropel (S_2), and usually has two distinct calcareous ooze layers at the top separated by hemipelagic mud; it is sometimes difficult to identify this cyclothem, but the well-defined calcareous layer (C_3) of the underlying third cyclothem delineates the base of cyclothem 2. The third (cyclothem 3) shows a well-developed sapropel layer (S_3) but, unlike cyclothem 1, usually includes a reduced proportion of turbiditic layers below the sapropel. This cyclic sequence varies from core to core in terms of thickness and absence of some sediment types.

The age of regionally important stratigraphic horizons in the three cyclothems is determined on the basis of radiocarbon dating (Figs. 2, 3 and Table 1). From top to bottom, the upper calcareous ooze (C_1) is about, or somewhat younger, than 2,700 yr b.p.; the oxidised layer (OL_1) is about 5,700 yr b.p.; the upper sapropel (S_1) ranges in age from about 6,600 to more than 8,200 yr b.p.; the calcareous ooze (C_2) top of the second cyclothem is dated about 17,000–19,000 yr b.p.; the second sapropel (S_2) is about 23,000–25,000 yr b.p.; the calcareous ooze (C_3) top of the third cyclothem is dated about 28,000–33,000 yr b.p.; the sapropel (S_3) in this cyclothem is roughly dated at 38,000–>40,000 yr b.p. The top of the fourth cyclothem (C_4) is considerably older than the resolution of carbon-14 method; a maximum age of 55,000–58,000 yr b.p. is estimated based on its position relative to the overlying dated horizons (Fig. 3, log 1).

For calculation of sedimentation rates, the following ages in yr b.p. are assigned to the top of the regionally prevalent stratigraphic horizons: OL_1=5,700; C_2=17,000; S_2=23,000; C_3=28,000; S_3=38,000; and C_4=55,000–58,000. The sapropel dates correlate relatively well with radiocarbon-dated sapropel sections of some workers[11]. Our sapropel 2 and 3 dates, however, are much younger than those obtained by other techniques (Ryan[12], for example, attributes an age of about 80,000 yr b.p. to S_3 which is not consistent with our findings).

It is useful to compare the combined litho- and chronostratigraphical sequence with generalised eustatic sea-level curves (modified after refs 19–21). The correspondence of cyclothems 1 and 2 with the Bloom *et al.* (1974) data is generally good, while the cyclothem 3 correlation is not so clear cut. The younger, more reliably dated cyclothems 1 and 2 are initiated with slight offset at times of maximum low and high sea levels (Fig. 3). Consideration of the climatic curve based on the Camp Century ice core[22] reveals a good correspondence for cyclothem 1 (warming after the Late Wisconsin); cyclothem 2 correlates with the Wisconsin II, between the Plum Point climatic maximum and the beginning of the Holocene rise; cyclothem 3 seems to correspond well with the Middle Wisconsin between about 30,000 and 58,000 yr b.p. including both Plum Point and Port Talbot maxima (D. J. Colquhoun, personal communication).

Calcareous ooze layers, consisting of abundant calcareous nannofossils and Mg-calcite in the form of poorly defined crystals, seem to develop at times of high and low sea-level stands, while the dark organic-rich sapropel layers accumulated during phases of rising as well as lowering sea level (Fig. 3). The relationship between lithology and the Camp Century and eustatic curves suggests the following: C_1 at about 2,700 yr b.p. may have been deposited during a time of slight sea-level lowering and probably cooler (*Littorina*) conditions than earlier. S_1 occurs almost 2,000 yr after climatic amelioration, and generally within the climatic optimum, at a time when the rate of Holocene sea-level rise had generally decreased. C_2 was deposited before the increase in temperature that resulted in the Holocene rise and probably during a time of slight temperature decrease which may also have been reflected in a slight sea-level lowering. S_2 may correspond to a warmer phase of the Late Wisconsin, and at about the same eustatic position as that of sapropel S_1, except for generally lower temperatures. C_3 occurs during a cooling phase from the Plum Point interstadial, with perhaps lowering sea level and temperatures. S_3 seems to occur close to the climax of a warmer period. The calcareous top of underlying cyclothem 4 may have accumulated during a cool phase leading into the Port Talbot interstadial (D. J. Colquhoun, personal communication). In this scheme hemipelagic and turbiditic sedimentation should prevail on the Cone at times of maximum sea-level changes when major delta front migration and Nile input alterations would be expected.

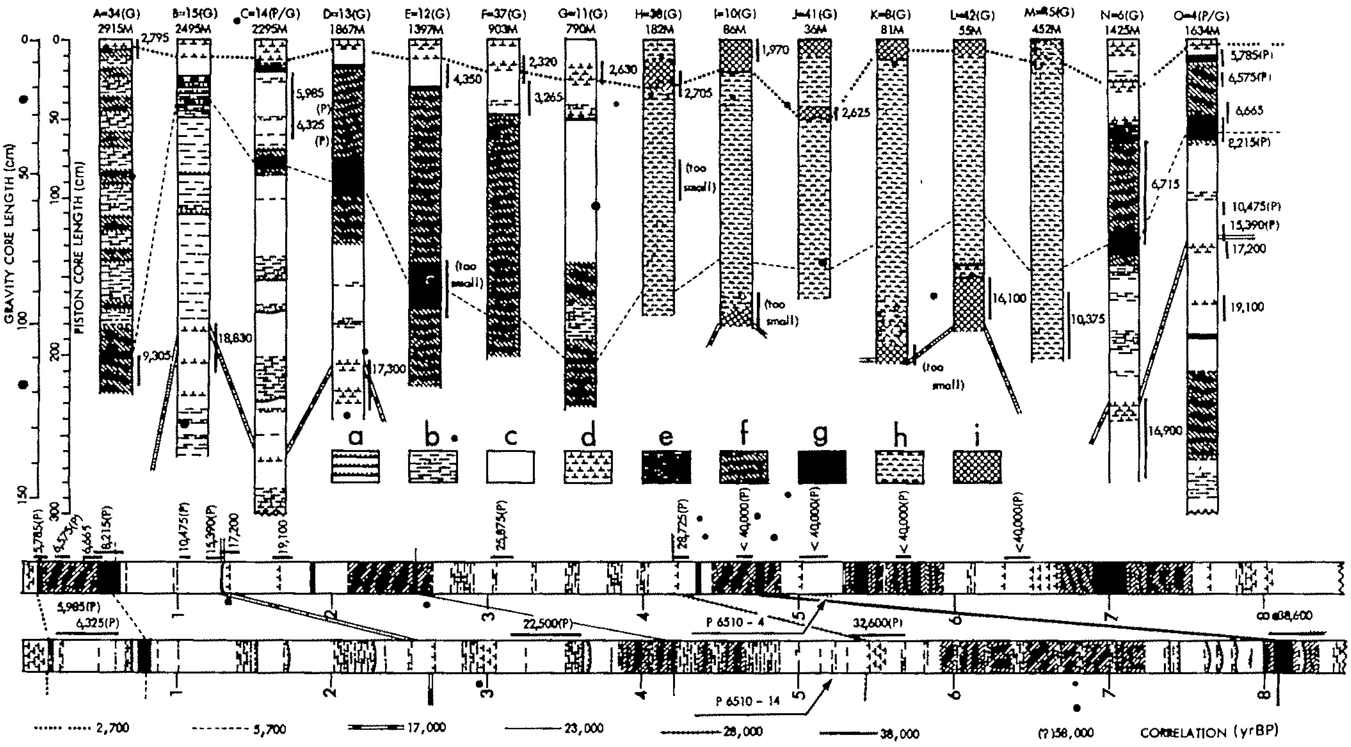

Fig. 2 Correlation of Nile Cone and Egyptian Shelf cores along two transects (position in Fig. 1) based on lithology and radiocarbon dating. Cores P6510-4 and -14 serve as types for defining the Late Quaternary stratigraphy in the southeastern Mediterranean. *a*, Turbiditic sand and silt; *b*, turbiditic mud; *c*, hemipelagic mud; *d*, calcareous ooze; *e*, oxidised layer; *f*, organic ooze and protosapropel; *g*, sapropel; *h*, grey (shallow water) mud; *i*, coarse calcareous sand and gravel. G, gravity core; P, piston core, p, pilot gravity core.

Sedimentation rate variations in time and space

The thickness measured between the major dated horizons in cores shown in Fig. 2 serves as a means to calculate sedimentation rates during six time periods in the late Quaternary. Depositional rates are contoured on six charts (Fig. 4) using a geometric scale to emphasise regional variations; the accuracy of contour line positions has been verified by supplemental lithofacies data from 27 Nile Cone cores collected on RV Chain 1975 cruise 119 (D. A. Ross and C. Summerhayes, personal communication). The charts reveal significant changes in sediment dispersal in the study area from the Early Würm to the present.

Low sedimentation rates are recorded during the oldest phase for which we have reasonable chronological control, that is, from about 58,000 to 38,000 yr BP (C_4 to S_3). The chart depicts minimal rates (about 1–16 cm per 1000 yr) since it is based on a limited number of cores which are long enough to recover the C_4 layer The rates may be higher than shown if the age of the C_4 layer is younger than the 55,000–58,000 yr BP postulated A broad lobe of relatively rapid sedimentation is observed on the upper Cone seaward of both Rosetta and Damietta branches (somewhat higher values occur off the Rosetta Branch). Low values occur elsewhere on the Cone, with lowest values recorded in the vicinity of the Eratosthenes Seamount. Deposits in this earliest time span considered here probably accumulated during several sea-level oscillations (Fig. 3) possibly related to the Port Talbot maximum[22]. We speculate that the calcareous ooze layer C_4 (base of cyclothem 3) was deposited during a low eustatic phase (termed the Early Würm[20], although no universally accepted sea-level curves are compiled for this period) before an interstadial (Plum Point) at about 30,000 yr suggested by some workers[19,21].

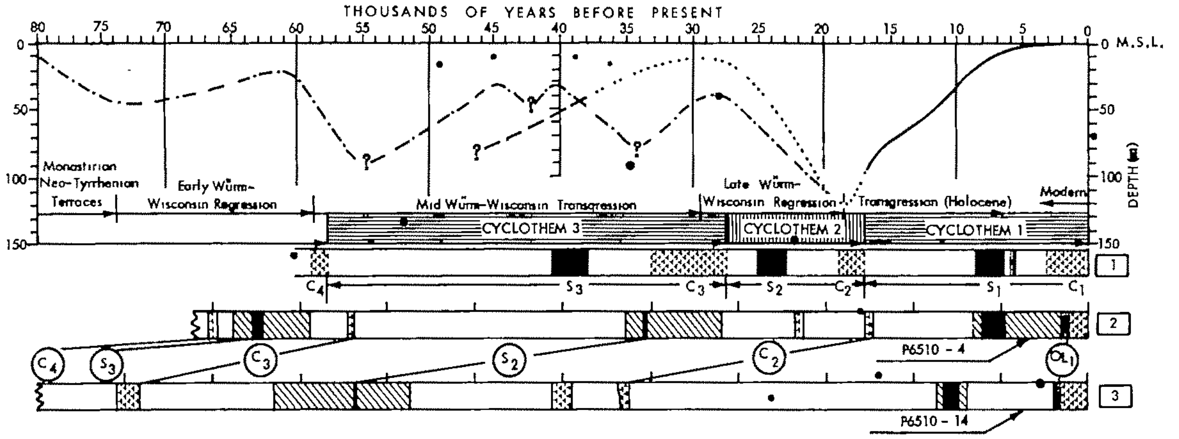

Fig. 3 Correlation between two Nile Cone stratigraphic type cores (logs 2, 3) and a generalised eustatic sea-level curve modified after refs 19–21. Log 1 shows the age of stratigraphic horizons and the upper three cyclothems in thousands of years before present; also shown is the position of the upper three cyclothems relative to sea level. OL_1 = oxidised layer above uppermost sapropel sequence; C_1, C_2, C_3, calcareous ooze layers; S_1, S_2, S_3, sapropel layers; · · · · · —— Curray[19]; — — — Fairbridge[20]; · — · — · Bloom *et al.*[21].

The depositional patterns are more distinct during the period between about 38,000 and 28,000 yr (S_3 to C_3). Rates range from about 1 to 26 cm per 1,000 yr, and most of the area is covered by sediment accumulating more slowly than 8 cm per 1,000 yr. A lobe of >16 cm per 1,000 yr is depicted on the Cone seaward of the Rosetta Branch; high values also occur off the Israel–Lebanon margin. This pattern developed either during a general rise in sea-level preceding, and perhaps coinciding with, the interstadial at about 30,000 yr BP (refs 19 and 20), or during a rising–lowering eustatic cycle[21] (Fig. 3).

Rates between about 28,000 and 23,000 yr BP (C_3 to S_2) range from 1 to >65 cm per 1,000 yr, and are generally higher throughout most of the region than in the previous period, that is almost half of the region is covered by sediments accumulating at >16 cm per 1,000 yr. Two well-marked lobes (>32 cm per 1,000 yr) occur seaward of both branches of the Nile; particularly high values are localised off the Rosetta Branch. This pattern evolved during lowering of sea level and reflects a steady increase of freshwater discharge and sediment supply from the Nile in response to climatic changes affecting the headwaters

Depositional changes are noted between about 23,000 and 17,000 yr BP (S_2 to C_2). The rates range from 3 to >49 cm per 1,000 yr, and at this time well over half of the area is covered by sediments deposited at >16 cm per 1,000 yr, reflecting the enhanced transfer of material on to the upper Cone sectors Patterns off the Nile are not distinct; low values occur off the Sinai, and two high sedimentation belts occur on the lower Cone, one extending SSW from Lebanon south of the Eratosthenes Seamount, the other (represented by three isolated areas) between the base of the Cone and the Mediterranean Ridge. The patterns, showing the highest rates over the largest areas, are related to the last major eustatic low stand when prodeltaic deposition extended seaward across the subaerially exposed shelf and large amounts of sediment were transported directly on to the slope. Mass gravity as well as hemipelagic and related suspension transport mechanisms were involved during this period of marked progradation.

From about 17,000 to 5,700 yr BP (C_2 to OL_1) depositional patterns once again become better defined. Sedimentation rates range from 3 to >88 cm per 1,000 yr; about a third of the Cone surface is covered by sediment accumulating at more than 16 cm per 1,000 yr, and half by 8 cm per 1,000 yr The highest rates appear channellised on the upper Cone and slope, and a relatively narrow NW-trending lobe extends from the Rosetta Branch towards the eastern part of the Herodotus Basin plain. An east–west zone of low sedimentation (<8 cm per 1,000 yr) forms on the Cone seaward of the Damietta Branch The more restricted extent of high sedimentation off the delta reflects a progressively decreased input of prodeltaic clastics paralleling the landward shift of the Nile depocentres during the rapid sea-level rise. This last major eustatic shift resulted in the proportionately increased role of hemipelagic sedimentation and diminished terrigenous influx from the Damietta sector of the delta

Sedimentation rates decrease further during the period between 5,700 yr BP and the present (OL_1 to C_1). Rates range from about 1 to 36 cm per 1,000 yr, and most of the Cone is covered by more slowly accumulating sediments (<8 cm per 1,000 yr). The highest rates occur on the shelf and upper slope, at the foot of the Cone, at the eastern section of the Herodotus Basin plain, and in the Eratosthenes Basin plain. The general decrease of rates away from land and absence of downslope channellisation patterns reflect the primary role of hemipelagic sedimentation and reduction of turbiditic mud incursions accompanying the stabilisation of sea level at about its present stand. The large east–west belt of low sedimentation is amplified as a result of the diminished role of the deltaic point source and relatively greater importance of lateral suspensate transport and smoothing by predominantly east–west trending surface water flow above the Cone[23–25].

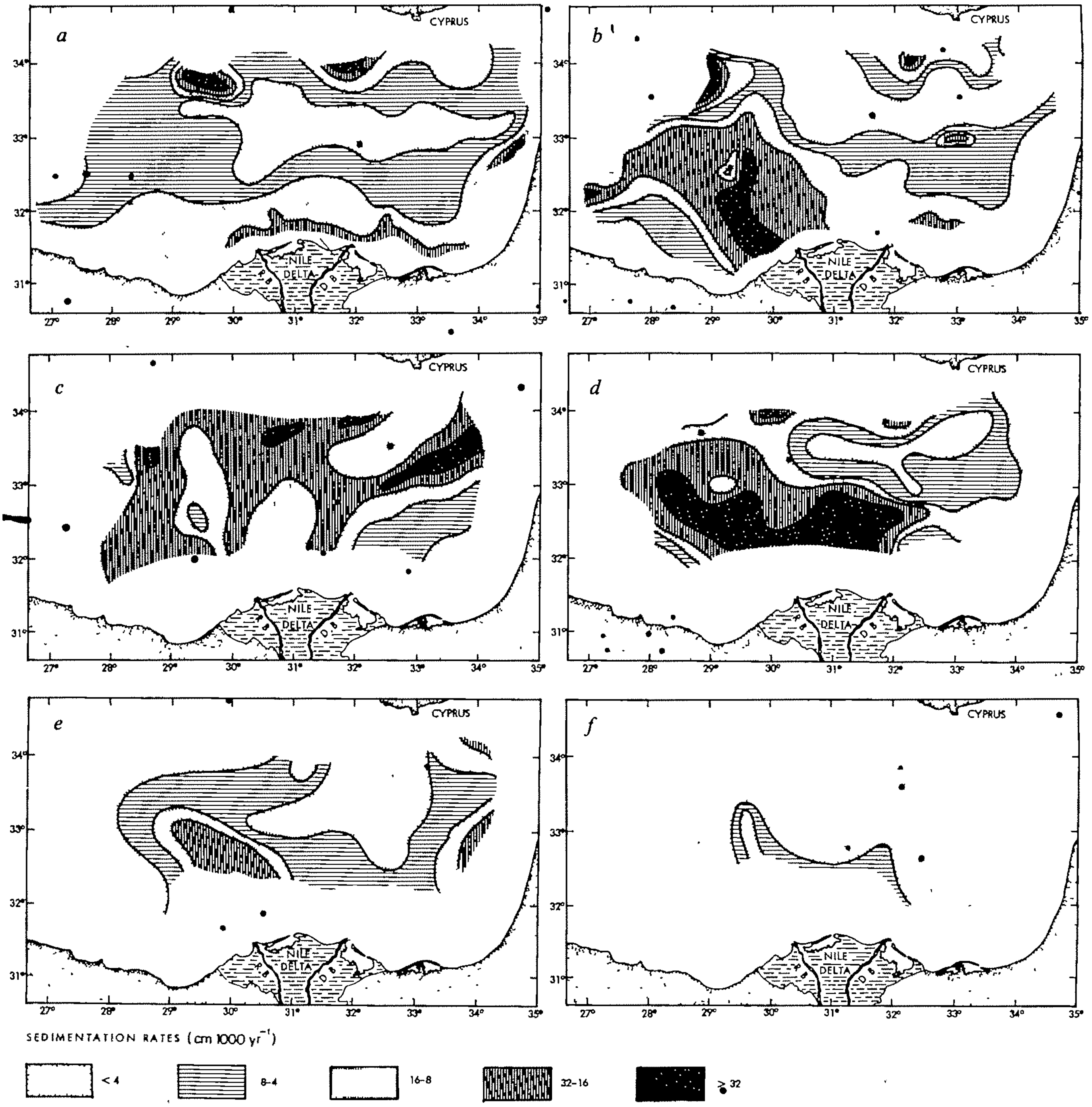

Fig. 4 Charts showing sedimentation rates (in cm per 1,000 yr) on the Nile Cone and adjacent areas during six periods in the Late Quaternary. The stratigraphic horizons used for correlation are shown in Figs 2 and 3. Explanation in text. *a*, OL_1–C_1 (5,700–present); *b*, C_2–OL_1 (17,000–5,700); *c*, S_2–C_2 (23,000–17,000); *d*, C_3–S_2 (28,000–23,00); *e*, S_3–C_3 (38,000–28,000); *f*, C_4–S_3 (58,000–38,000). Bloom *et al.*[31].

Discussion

The orderly and repetitive nature of Late Quaternary sediment types on the Nile Cone and adjacent south-eastern Mediterranean reflects the interplay of sedimentation with highly variable climatic[1,26,27], physical oceanographic[28] and biogenic against terrigenous input factors in a quasi-enclosed silled basin. The core data presented here establish a time-framework for the upper three cyclothems, and provide support for the previously suggested hypothesis that the cyclic nature of major lithofacies in this region is a direct response to climatic factors[17]. Specifically, we show that cyclothems correspond closely in time with either major eustatic rise or lowerings of sea level, and that the beginning of each new cyclothem appears slightly offset (delayed) from the beginning of an oscillation (Fig. 3).

The dated calcareous ooze layers were deposited primarily during cooling temperatures and lowering sea-level stands. Several radiocarbon-dated samples of magnesium-rich layers in other eastern Mediterranean cores (ref. 29, their Table 3) correspond in age to Nile Cone calcareous ooze horizons C_2 and C_3 identified here. Similar calcareous ooze layers of about the same age also are recorded in the western Mediterranean[30]. The sapropels, on the other hand, accumulated during periods of rising or lowering sea level, and not at times of highest or lowest stands. Earlier work suggested they were deposited primarily during warming trends of climatic curves[12]; our study indicates they accumulated during early stages of temperature maxima. Sapropels record repetitive phases of stratification and near-bottom stagnation[5,15,28,31,32]. The general sapropel depositional model is characterised by (1) layering of water masses, (2) stagnation of bottom water with the formation of H_2S-rich zones and (3) seasonal (winter–summer) or periodical changes (eustatic-related upwelling and vertical mixing of water), all of which favour the development and preservation of varve-like bedding[15,33]. The oxidised layer found above the sapropels, the third cyclothem lithotype related to climatic and oceanographic phenomena, marks a return to oxygenated bottom-water conditions.

The time and spatial changes in sedimentation rates show the degree to which deposition has been directly affected by the Quaternary climatic–eustatic oscillations. The period of overall highest sedimentation occurred between about 28,000 and 17,000 yr BP; it is during this time span (last major Wurm regression) that cyclothem 2 was deposited. As sea level dropped and Nile River discharge patterns altered, an increasing volume of resuspended sediment was transported across large sectors of the delta front, including both the Rosetta and Damietta branches, and moved seaward directly on to the Cone. During this period sedimentation rates over large areas of the Cone are roughly equivalent to the high, long-term average rates (>30 cm per 1000 yr) previously postulated[3,14] for this and other submarine fans[34–37] during the Pleistocene. At times of rising sea level, sediment patterns on the Cone (cyclothems 3 and 1) show more restricted zones of sediment influx localised seaward of the Rosetta Branch, and an extensive east–west belt of low sedimentation over the central and eastern sectors of the Cone The latter records the diminished input from the delta and the relatively increased importance of suspension processes and regional smoothing by currents responding to large scale water mass flow over the Cone. As might be expected, lowest depositional rates are recorded at times of high sea-level stands, such as exists at present when the influx of the Nile is at a minimum due to the submergence of the Egyptian Shelf, landward regression of the prodelta lobe away from the shelf edge, and reduced freshwater discharge.

The most important zone of accretion on the Cone during the total time span (≃58,000 yr) considered here forms a SE–NW lobe which extends seaward of the Rosetta Branch toward the eastern part of the Herodotus Basin plain. The broad, highly dissected SE–NW trending belt east of this depositional tongue and north of the Damietta Branch[1] does not seem to have had a significant role in downslope channellisation of sediments during the Late Pleistocene–Holocene and even may have served as a barrier to the northward and northeastward transfer of clastics from the delta.

The Late Quaternary cyclothemic patterns of the type defined here occurred through much of the earlier Pleistocene, and possibly Pliocene, development of the Nile Cone and, if carefully documented, these older cycles may identify climatically-induced changes back in time. The stratigraphic generalisations can also serve to predict the future evolution of this submarine fan. In this respect, the emplacement of the Aswan High Dam and cessation of surplus Nile flood water discharging through the delta mouth is bringing to completion a process initiated with the rapid Holocene rise of sea level, that is, the reduction of seaward transported riverborne sediment. The present artificial 'closing-of-the-faucet' phenomenon has markedly altered the nature of suspended sediment and nutrients seaward of the delta and reduced the volume of material likely to reach the shelf edge. Immediate predictable results include lowered depositional rates on and beyond the shelf and a broadening of the east–west belt of low accretion on the Cone

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Monitoring the marine environment through sedimentation

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The argument is advanced that the interception of particles settling within the water column provides a means of monitoring and understanding basic ocean processes. Geochemical and biological measurements on precisely dated bottom sediments and on preliminary recoveries from particle interceptor-traps provide supporting evidence.

ATTEMPTS to monitor and comprehend complex natural systems such as coastal seas and central oceans are characterised by the study of accessible parameters. These parameters are *a priori* presumed to be of importance, and it is further implied that a synthesis of accumulating information will eventually provide a sufficient description of the entire system.

Major problems in scaling arise if the relationship of discrete sampling to the continuous and long-term operation of natural systems is considered. In the case of the ocean this usually translates into the intermittent and non-synoptic measurement of a number of classic factors (such as temperature, salinity, major nutrients, chlorophyll, certain phytoplankton and zooplankton species, nekton and currents). These measurements are made at a number of point locations usually for very short periods, and extrapolations to the seasonal and longer-range character of the ocean are attempted In such a situation, though there occasionally may be an overwhelming number of samples, there is seldom sufficient information.

An alternative to increasing the number of measurements to gain sufficient data is to enlarge the scale of each measurement, making each observation representative of a relatively large area and significant period of time.

High-resolution bottom sediment records

Study of marine bottom sediments as a record of the overlying ocean is a long-standing and continuing[1] approach to increasing the scale of measurement. The apparent advantage of bottom sediment study is the access to material representative of prehistoric times, and were the open ocean and coastal seas unaffected by human activities, this would be the dominant attraction. If we are concerned with contaminant metals and hydrocarbons in the present marine environment, however, this proposition requires elaboration. The coastal sea lying directly off southern California is an important and convenient region on which to centre discussion.

The four nearshore coastal basins (Santa Barbara, Santa Monica, San Pedro and San Diego) adjacent to the southern California coast fall midway along a broad southward-flowing body of water known as the California Current[2]. The Santa Barbara, Santa Monica and San Pedro basins have impoverished bottom fauna zones as a direct result of their sills intersecting a dissolved oxygen minimum in the water column[3], and as a consequence, a virtually undisturbed sequential record of sedimentary phases accumulates. These basins, particularly Santa Barbara in the north, contain the greatest amount of information to be found in any sediment deposit, and this information can be resolved on time scales approaching 1 yr.

Utilising techniques that include box-coring devices that consistently obtain the exposed sediment surface, verification of surface sediment recovery through the presence of excess ^{228}Th, and stratigraphic dating by varve and ^{210}Pb chronology[4], detailed historical studies have been performed. In the Santa Barbara Basin sediments, these studies include common lead and its isotopic ratios[5], mercury[6] and the halogenated hydrocarbons, DDE and PCB (ref. 7) Metals including Pb, Cr, Cd, Zn, Cu and Ag have been analysed in the surface and near-surface sediments of the three suboxygenated basins[8]. Such studies not only provide a considerable perspective into baseline levels and subsequent anthropogenic increases of certain elements (Fig. 1*a*) but also most certainly indicate the sensitivity of sediments as an index to changes in the chemistry of the marine environment.

The range of factors available for examination in sediments is not limited to chemical considerations. Formation of a detailed chronological framework in sediments such as those of the Santa Barbara Basin gives a view of the long-term operation of the overlying biological system. This view is limited both by incompleteness of the sediment record and by the investigational effort needed to decipher the record, but is nonetheless remarkable. As an example, it has been found that fish scales of the two historically dominant pelagic species (Pacific sardine and northern anchovy) accumulate in the Santa Barbara sediments, and the accumulation is closely and directly related to fishery-derived population estimates for the entire southern California region[11]. This relationship leads to an estimate of the variation of fish biomass in past times (Fig. 1*b*) and also suggests that presently depositing scales are a likely index to present productivity.

A second instance can be drawn from the study of diatom assemblages in these same sediments. If the combined absolute abundances of a number of diatom species, thought to be indicative of warm subtropical waters, are compared through time to an index of California summer surface temperature, it seems that the two records are significantly and directly related[12].

It should be noted that there is internal evidence that information derived from basin sediments applies to a considerable areal scale. Studies of the relationship of regional rainfall patterns to sedimentation (A.S. and P.A.C., in the press) demonstrated that individual laminae in the Santa Barbara sediments often have sufficient morphological character for them to be traced between cores 5 km apart. This is one indication that horizontal forces operating in the water column can effectively disperse and homogenise sediment over large areas; furthermore, in the basin regions off southern California the

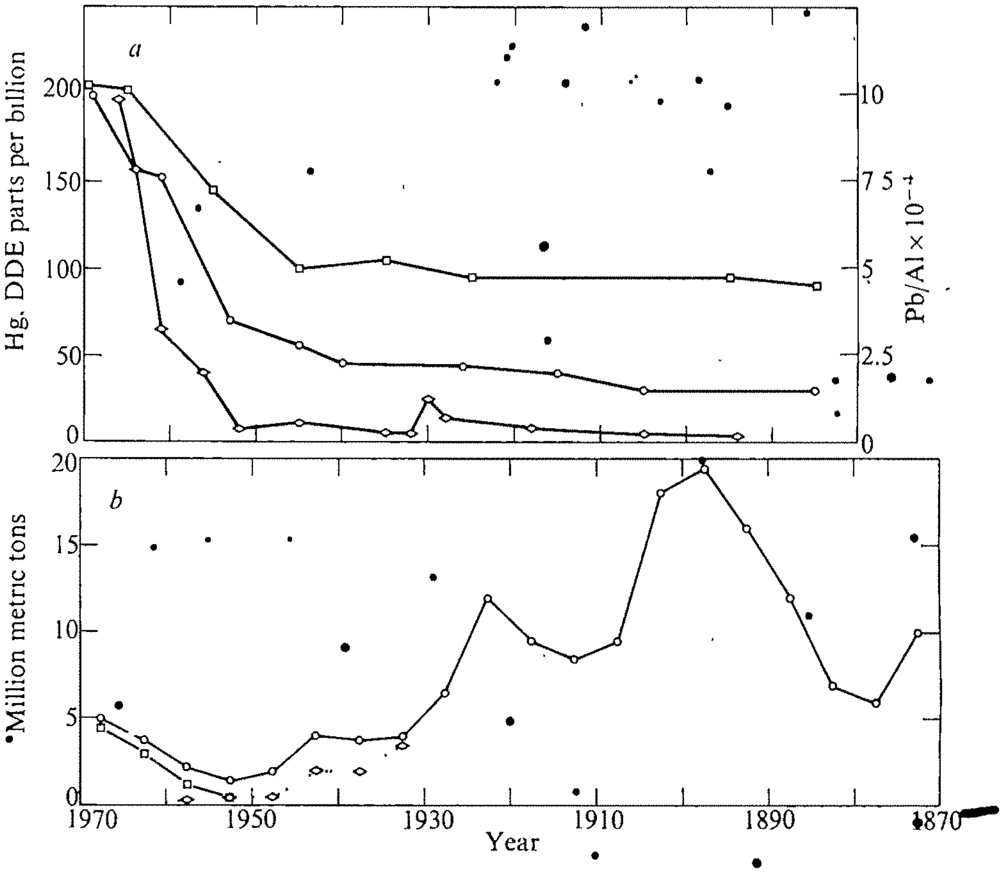


Fig. 1 Time series records from dated sediments off California. *a*, Lead[5] in the San Pedro Basin (○) and mercury[6] and DDE (ref. 7) in the Santa Barbara Basin (□ and ◇ respectively). The salt-corrected dry sediment concentrations of Hg and DDE are given in parts per billion, and Pb as a ratio of Pb/Al $\times 10^{-4}$ Other metal values for the Santa Barbara Basin 1970–71 surface sediments[8] are: Mn/Al $\times 10^{-4}$, 51; Fe/Al, 0.63; Ni/Al $\times 10^{-4}$, 7.3: Cr/Al $\times 10^{-4}$, 28; Zn/Al $\times 10^{-4}$, ~25, Co/Al $\times 10^{-4}$, 3.4, V/Al $\times 10^{-4}$, 44; Cu/Al $\times 10^{-4}$, 8.3; and Pb/Al $\times 10^{-4}$, 6. Corresponding values for metals in sediment recovered from a particle interceptor-trap deployed 100 m below the surface in Santa Barbara Basin during October 1971 are: Mn/Al $\times 10^{-4}$, 63, Fe/Al, 0.64; Ni/Al $\times 10^{-4}$, 4.5; Cr/Al $\times 10^{-4}$, 22; Zn/Al $\times 10^{-4}$, 107; Co/Al $\times 10^{-4}$, 1.5; V/Al $\times 10^{-4}$, 16; Cu/Al $\times 10^{-4}$, 13; and Pb/Al $\times 10^{-4}$, 16. *b*, Northern anchovy biomass estimates[9] (□), Pacific sardine biomass estimates[10] (◇), and combined pelagic fish biomass estimate (northern anchovy, Pacific sardine and Pacific hake) (○) derived from fish scale deposition in Santa Barbara Basin[11].

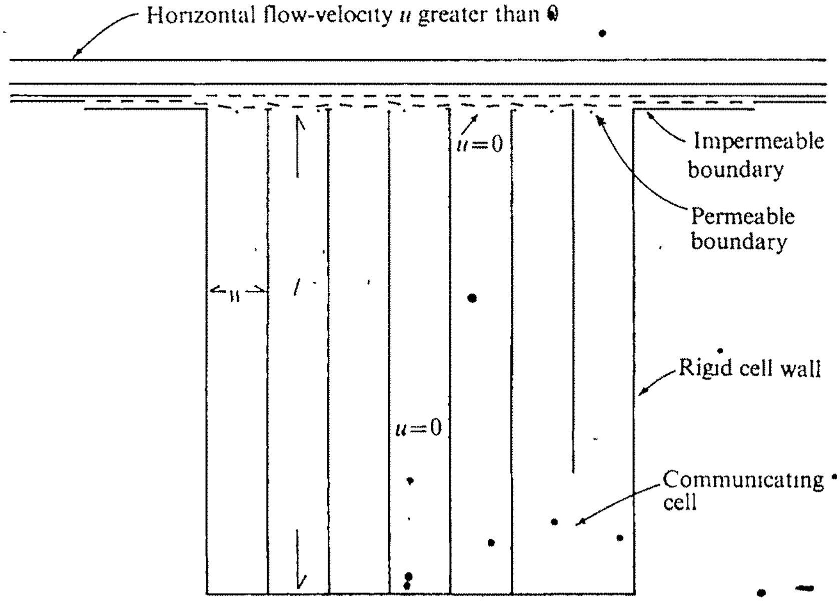
parameter values found in a reliable and documented core are generally good measures of any other reliable core recovered from the same basin, and overall the sedimentation of biogenic and terrestrial debris provide sensitive indices of regional climate, productivity and pollution.

Particulate sediment in the water column

If the bottom of the sea everywhere were similar in character to the Santa Barbara Basin, there would be little need of further elaboration. Varved sediment, however, is quite rare and Santa Barbara is the only known location in the coastal seas off the mainland United States. Although there are significant occurrences of laminated sediments within the Gulf of California, Mexico and the coastal seas off Peru, South Africa and Pakistan–India, the bottom sediment records that do exist are for the most part thoroughly mixed by benthic organisms and have low to moderate rates of deposition (1–100 cm per 1000 yr). Resolution is thereby limited in such accumulations to time scales greater than 100 yr.

Direct collection of sediment particles in the water column is a means of obtaining information reaching the sediment surface, with potentially higher resolution than most bottom sediment records. Particulates may be directly filtered from the water column by nets[13] or from water

Fig. 2 Empirical configuration of particle interceptor-traps Cross-section diagram of closed cell and communicating cell array with horizontal water flow. Provided cells are closed at the bottom (or in the case of communicating cells the length (*l*) is at least four times the width (*w*)) and the cell walls are rigid, a quasi-stable boundary regime will be maintained at the cell mouths. Steady horizontal flow (*u*) will exhibit some drag effects to about 1/5 *w* above the cell mouth; however, depending on the current velocity, penetration of drag-induced vertical movement ceases at 1/5 *w* or less into the cell mouth. For cell widths on the order of 1 cm or less, even with reasonably high current velocities (30 cm s^{-1} highest observed in tank), frictional drag will not induce horizontal currents or vertical turbulence within the cells In effect the water inside the cells, though still in open diffusional continuity with the overlying flow, is isolated, and only settling particles (and small organisms capable of swimming) may enter and accumulate in the cells. Since the cells are isolated, abiotic agents can be incorporated and maintained as a relatively denser brine at the bottom of the cells to minimise post-depositional grazing and bacterial effects.



samples[14]. Such collection provides a measure of particle concentration but suffers from a low yield and the high variability of non-synoptic point sampling. It seems that systematic collection of particulates by filtration would be a useful but limited monitoring strategy.

Interception and collection of sediment in gram amounts and direct assessment of particle flux is possible through the emplacement of particle interceptor-traps within the water column. In conditions of horizontal current flow these devices can, in effect, locally create a hydrodynamic environment[15] similar to that found at the sea floor and thereby induce sedimentation. The critical features of a sea-floor boundary region can be produced by a horizontal, open-top/closed-bottom cellular array (Fig. 2).

Table 1 Dry sediment accumulation in particle interceptor-traps (615 cm^2)

Region		Time down (h)	Dry sediment yield (g cm^{-2} yr^{-1})	Yield as fraction of long term bottom sedimentation rate*
Santa Barbara Basin 34°14′N; 120°01′W depth 580 m				
May 1969	150 m below surface	50	0.02	0.22
	30 m above bottom	50	0.03	0.33
December 1969	10 m above bottom	85	0.17	1.90
October 1971	100 m below surface	840	0.08	0.88
	10 m above bottom	840	0.09	1.00
October 1973	150 m below surface	810	0.03	0.33
	10 m above bottom	810	0.06	0.66
San Pedro Basin 33°30′N; 118°22′W depth 890 m				
May 1972	150 m below surface	640	0.01	0.36
	10 m above bottom	640	0.01	0.36
Soledad Basin 25°15′N; 112°41′W depth 520 m				
May 1969	150 m below surface	50	0.05	1.28
	180 m below surface	50	0.02	0.51
	40 m above bottom	50	0.03	0.77
	10 m above bottom	50	0.03	0.77
October 1973	150 m below surface	590	0.01	0.27
	10 m above bottom	590	0.30	7.70

*Bottom sedimentation rates[22]: Santa Barbara, 0.089 g cm^{-2} yr^{-1}; San Pedro, 0.028 g cm^{-2} yr^{-1}; Soledad, 0.039 g cm^{-2} yr^{-1}.

Sediment trap configurations that have been used in lakes[16] are essentially open-top standing tubes 5–15 cm in diameter. Such configurations, and indeed many others, are well suited for particle collection provided there is little or no horizontal current movement and the particles have a relatively fast settling velocity. In the sea, where near-bottom flow may average 1–5 cm s^{-1} and on occasion exceed 25 cm s^{-1}, the cellular grid array would seem to be more in harmony with the conditions of sedimentation. The situation can perhaps be visualised if the path of a diatom frustule settling at about 1 m h^{-1} and being carried along in a 2-cm s^{-1} horizontal current flow is considered. In this case the tangent path of the diatom will form a vector angle from the horizontal of less than 1°. Furthermore, the grid array configuration facilitates the practical engineering of collecting surfaces of 1 m^2 or greater necessary to yield sufficient material in the relatively slow sedimentation regime of the ocean[17].

It must be stressed that grid arrays acting as particle interceptor-traps provide a measure of particle flux and, as such, cannot everywhere provide a meaningful measure of sedimentation rates. In nearshore and shallow (less than 100 m) regions there may be considerable resuspension and temporary deposition. Particle interceptor-traps placed near the bottom in such areas can only be used to qualitatively describe sediment in transit. Quantitative collection can ideally be carried out in the waters lying above deeper (> 250 m) basins and gently sloping regions of permanent sedimentation.

Alternatively, the use of particle interceptor-traps is not limited solely to the estimate of particle flux in a long term monitoring context. For example, if concurrent with the interception of particles, the in-the-water particle concentration is measured, then, provided the concentration is reasonably stable, dividing the particle flux ($ML^{-2}T^{-1}$) by the particle concentration (ML^{-3}) gives an estimate settling velocity (LT^{-1}).

Since an initial attempt[18], we have used particle interceptor-traps of 615 cm^2 for preliminary studies off the coast of California These traps were routinely deployed on free drop systems[19] consisting of a radio and sight mast, connecting lines of varying lengths, subsurface flotation packs, particle interceptor-traps, timed-release devices and ballast. The system is released from the ship in the stated order and settles to the bottom with traps positioned from 10 m above the bottom to 100 m below the surface. After a preset time, the release closes the traps and releases the ballast or radio mast allowing the system to rise to the surface. In nearshore and other areas of good navigation a less expensive though more vulnerable system can be arranged with a surface marker buoy. Alternatively, an expensive though relatively more efficient arrangement is possible using telemetering acoustic recall devices.

Results that may be useful in considering particle interceptor-traps as a monitoring tool are as follows. The dry sediment yields for a number of drops are given in Table 1. The values for 100–150 m below the surface traps were 0.01–0.08 g cm^{-2} yr^{-1}. These projected values range from 0.22 to 1.28 times the underlying long term bottom sedimentation rates. The corresponding values for the

10–40 m above the bottom traps are 0.01–0.30 g cm^{-2} yr^{-1} and 0.33–7.70 times the bottom sedimentation rate. One of the extreme bottom values (Santa Barbara, December 1969) has been interpreted in conjunction with hydrographic observations as being due to a density flow deposit (turbidity current)[20]. The metal composition of sediment recovered from the Santa Barbara, October 1971 drop is given in the legend to Fig. 1*a*. Although these traps were not especially cleaned for metal investigations, with the exception of zinc, comparison of trap metal values and metal values of surface sediment in the same year are in general agreement.

Major seasonal differences in the aspect of intercepted sediment have been observed. In the Santa Barbara Basin, winter precipitation and storm wave activity lead to distribution and sedimentation of land detritus. Direct transportation of land detritus to the central basin region has been inferred as likely from the collection at 100 m of an essentially clay–silt sediment in the October 1971 trap. This trap was deployed 2 weeks before a substantial and first rainfall of the season (previous rainfall 6 months before) and was recovered 2 weeks after. Delayed distribution of land-derived detritus has been documented[21]. Sediment initially deposited on the shelf adjacent to the basin is progressively mobilised by subsequent storm waves and disperses towards the central basin on pycnocline surfaces near the 100-m depth. Sediment observed accumulating in traps deployed during the rainy season closely resembles the bulk composition of the Santa Barbara bottom sediments in that it is made up of clay flocculates with abundant silt particles. In contrast to winter deposition, material accumulating in the traps during the spring and summer seasons may on occasion differ substantially in appearance As an example, in May 1969 in both the Santa Barbara Basin and the Soledad Basin almost the entire fallout was made up of faecal pellets. The pellets contained no recognisable microfossil skeletal fragments in any of the size fractions (Table 2), and the percentage of organic carbon in these samples (18–20%) would indicate a transfer to the bottom of both basins at that time of about 0.2 g carbon m^{-2} d^{-1}.

In all seasons abundant and representative biogenic debris accumulate in particle interceptor-traps. Fish scales, pelagic molluscs, foraminifera, radiolarians, silicoflagellates, diatoms and coccoliths have all been observed. As a specific example, the group of warm-water indicator diatoms referred to in the bottom sediment discussion were well represented in both the surface and bottom traps deployed in October 1973. It is not possible to relate isolated monthly trap measurements to general temperature information, as little is known about seasonal differences in species abundance and productivity of diatoms off California. But the flux of diatoms in October 1973 projected for the entire year falls within the measured bottom sediment distribution. Furthermore, it should be stressed that all the above results are preliminary and indicate that particle interceptor-traps must be calibrated through year-long studies before they can be truly used as quantitative instruments in the marine environment.

Table 2 Estimated numbers of faecal pellets accumulating in particle interceptor-traps (50-h collection)

Region		Particle size range (μm)				
		>500	500–246	246–124	124–24	Total
SantaBarbara Basin						
May 1969	150 m below surface	600	6,000	6,000	30,000	42,600
	40 m above bottom	40	2,200	5,000	24,000	31,200
Soledad Basin						
May 1969	150 m below surface	0	50	6,600	~33,000	~40,000
	10 m above bottom	35	20	2,800	~20,000	~23,000

Monitoring the sea

It is difficult to devise a predictably successful monitoring strategy in the marine environment, for we consistently minimise the intricate nature of the sea and exaggerate our capabilities. Few programmes have ever set out to study persistently and record a substantial part of any marine environment; none have lasted intact much more than a decade.

If we are to assess the effects of future assaults on the seas and oceans, we must begin now to record marine conditions. We must monitor the chemical condition of the sea, paying close attention to the pathways and fate of heavy metals, anthropogenic hydrocarbons and artificial radionuclides. We must monitor the biological condition of the sea, trying to understand or at the least appreciate the fluctuations in productivity and species composition that do occur.

We feel that a unified monitoring of coastal seas and central oceans commensurate with available resources and a common responsibility for the future should soon exist[23]. Such an undertaking should involve a number of strategies. We contend that the interception of sediment particulates in conjunction with bottom sediment studies constitutes a major approach.

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Penicillopepsin from *Penicillium janthinellum* crystal structure at 2.8 Å and sequence homology with porcine pepsin

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The polypeptide chain of the acid protease penicillopepsin folds via an 18-stranded mixed β-sheet into two distinct lobes separated by a 30-Å long groove which is the extended substrate binding site. The catalytic residues Asp-32 and Asp-215 are located in this groove and their carboxyl groups are in intimate contact. Alignment of the amino acid sequence with that of pepsin shows regions of high homology.

ACID proteases are important in many diverse biological functions[1]. Fungal enzymes have been investigated as potential replacements for chymosin, the acid protease from calf stomach used in cheese production, owing to an ever increasing shortage of rennet. Acid proteases are also used in the preparation of fermented products from rice and soybeans. In mammals acid proteases function not only as extracellular digestive enzymes (pepsin, gastricsin) but also have a role intracellularly in protein turnover (cathepsins D and E (ref. 2)) and in the control of blood pressure (renin, the angiotensin-releasing kidney enzyme[3]).

Although chymosin has been used as an enzyme solution (rennet) for as long as 6,000 yr and although the first X-ray photographs taken of a protein crystal were those of pig pepsin[4], the study of the primary and tertiary structures of acid proteases has been slow. The complete amino acid sequence of only pig pepsin[5] is known and a preliminary communication[6] on its tertiary structure at 2.7-Å resolution has been published. Low resolution structures of two microbial acid proteases, one from *Rhizopus chinensis*[7] and the other from *Endothia parasitica*[8], have also been determined, but no detailed description of the polypeptide chain conformation for acid proteases has been published.

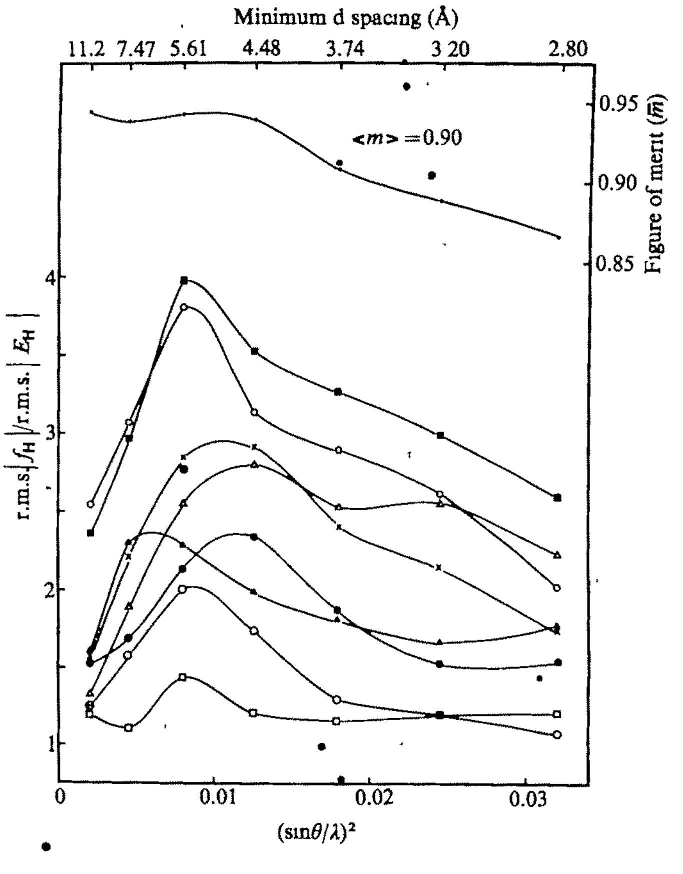
Penicillopepsin, the extracellular acid protease from *Penicillium janthinellum,* is an evolutionary homologue of pig pepsin and chymosin[9] as judged from a comparison of partial sequences. In addition, its specificity and catalytic mechanism are very similar to those of pig pepsin[10]. An aspartic acid residue important in the catalytic mechanism has been identified in a sequence which is nearly identical to one of the active site sequences of pig pepsin[9,11].

We present here the first high resolution structure of an acid protease in which the complete polypeptide chain can be traced unambiguously. The studies of the structures of the various acid proteases that are currently under way will demonstrate whether or not all these enzymes are homologous and belong to a single enzyme family such as the alkaline serine proteases[12–16]. Similarities and differences in their structures should also help to delineate specific mechanisms and substrate specificities. Our description of penicillopepsin at 2.8-Å resolution shows features which are likely to be common to many enzymes in this family.

Crystallography

Preliminary data for penicillopepsin crystals (monoclinic, space group C2, cell dimensions, $a = 97.38(8)$, $b = 46.62(3)$, $c = 65.39(6)$ Å, $\beta = 115.4(1)°$) from 2.5 M Li_2SO_4, 0.1 M sodium acetate, *p*H 4.4 were reported before[17]. A low resolution structure at 6 Å has also been described[18]. We used data collection and processing techniques described in the structural studies of the B protease from *Streptomyces*

Fig. 1 Phasing power of the eight heavy-atom derivatives used for the MIR phase determination of penicillopepsin. The ratio of r.m.s. $|f_H|$ to r.m.s. $|E_H|$ and the figure of merit ($\bar{m}$) are plotted as functions of $\sin^2\theta/\lambda^2$. ○, Uranyl acetate; △, $Pt(NH_3)_2Cl_2$; x double derivative (Pt + U); ●, mersalyl; □, K_2HgI_4; ■, $K_3UO_2F_5$; ▲, phenylmercuric acetate; ⊙, $IrBr_3$.



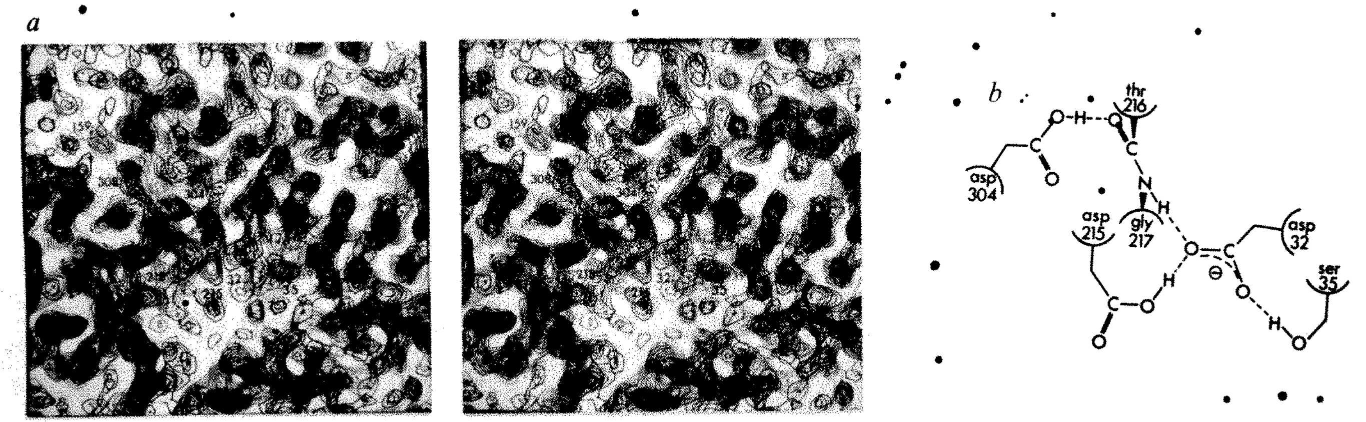


Fig 2 *a*, Stereoview of 10 sections (1-Å spacing) of the electron density map of penicillopepsin in the region of the catalytic site. The sequence numbers (see Fig. 3) are placed close to the side chains in this figure. The close contact of the carboxyl groups of Asp-32 and Asp-215 is in the central portion of the figure and the extended substrate binding groove lies below (in this figure) the active centre region. *b*, A tentative interpretation of the electron density in the vicinity of the active site of penicillopepsin. The carboxyl groups of Asp-32 and Asp-215 are approximately coplanar and share a proton as shown. Asp-32 is involved with two additional hydrogen-bond interactions, one to Ser-35, the other to the main chain NH of Gly-217. Asp-304 is an internally buried side chain and is involved with a hydrogen bonding interaction to the main chain C = O of Thr-216.

griseus[15,19]. The crystals of penicillopepsin were sufficiently stable to radiation damage in the X-ray beam to allow the collection of the complete 2.8-Å sphere of data (6,700 independent reflections) from a single crystal of the native enzyme and each isomorphous heavy atom derivative. The maximum loss in intensity of the monitor reflections was between 3 and 13% for the various crystals.

Eight heavy-atom derivatives were used to determine the phase angles for the reflections from the native crystals. The heavy-atom binding sites were identified either on difference Patterson maps or cross-phased Fourier maps which made use of the protein phases available from the 6-Å work[18]. Refinement of the heavy atom parameters was by the method of least-squares. For two derivatives, K_2HgI_4 and $IrBr_3$, the final heavy-atom model included two planar trigonal groupings of the HgI_3^- ion and $IrBr_3$. All heavy atom parameters, occupancies, temperature factors and positional parameters, behaved extremely well during the course of the least-squares adjustments with alternating cycles of protein phase angle determination. Table 1 shows the refinement statistics. Figure 1 shows the distribution of the ratio of heavy-atom scattering power (f_H) to lack of closure error (E_H) and of the mean figure of merit ($\bar{m}$) in ranges of $\sin^2\theta/\lambda^2$. The overall mean figure of merit for all observed data to 2.8-Å resolution was 0.90. It is clear from Fig. 1 and Table 1 that all eight heavy-atom derivatives provided useful phasing information to 2.8-Å resolution. The electron density map was computed from the 6,127 (91.4% of total) 'best' phases[20] with the measured native protein structure factor amplitudes. The high quality of the map may be judged from Fig. 2 by an examination of several sections computed in the region of the molecule containing the putative active site (Asp-32, Asp-215 in the pig pepsin numbering[5]).

Table 1 Heavy atom refinement summary

Derivative	Soaking time (d)	No. of sites*	Range A† (electrons)	Range B‡ (Å^2)	R_c§
1 mM $UO_2(C_2H_3O_2)_2$	7	3	11.5–22.6	6.9–18.4	0.512
5 mM $Pt(NH_3)_2Cl_2$	7	2	11.4–43.8	11.4–22.6	0.501
1 mM Pt + 1 mM UA	14	4	8.7–21.2	7.4–17.1	0.581
Mersalyl (saturated)	3	7	5.7–28.4	11.6–25.6	0.587
0.5 mM K_2HgI_4	3	5	5.4–27.6	5.3–20.7	0.626
5 mM $K_3UO_2F_5$	21	4	6.7–31.8	10.9–17.8	0.503
Phenylmercuric acetate (saturated)	7	7	6.6–42.7	9.1–17.9	0.569
2.5 mM $IrBr_3$	4	6	4.6–37.4	3.7–43.5	0.653

*Both the K_2HgI_4 and $IrBr_3$ derivatives had two sites which consisted of trigonal moieties (of HgI_3^- and $IrBr_3$, respectively).

†A is the site occupancy on an approximately absolute scale.

‡B is the isotropic temperature factor coefficient.

§$R_c = \Sigma||F_{PH} - F_P| - |f_H||/\Sigma|F_{PH} - F_P|$ summed over the centric data only.

Amino acid sequence

When the 2.8-Å resolution electron density map was computed, the primary structure was available in 16 fragments ranging in length from 3 to 112 amino acids[9]. In addition, there was tentative evidence for four overlaps. The map and the partial sequence data were used in conjunction to place 14 of the fragments in their proper order and to produce the sequence of penicillopepsin shown in Fig. 3. For two regions, 247–264 and 291–302, there was considerable difficulty in correlating sequence data with the electron density distribution. Residues 260–263 are consistent with the sequence of fragment PP6 in ref. 9. Residues 247–259 are consistent with much of fragment PP11 with the exception of the pentapeptide 249–253. In addition, the position of Cys-250 was not confirmed in fragment PP11. We have left residues 249–253 in brackets but the electron density in this region is consistent with the assignment of residues we have made based on the size, shape and spatial surroundings of these side chains. There is no doubt about the assignment of Cys-250 which is involved in the disulphide bridge 250–283. Eight residues from 295–302 satisfactorily account for the electron density in that region of the map and correspond in part to the C-terminal portion of fragment PP12 (ref. 9). Although residue 299 was chemically identified as Phe we have assigned Tyr to this residue based on the electron density associated with the side chain and the fact that a dipeptide Tyr–Leu was obtained in relatively high yield (11%) from a thermolytic digest.

Figure 3 also shows a possible alignment of the penicillopepsin sequence with that of pig pepsin[5]. A final alignment must await comparison to the tertiary structure of pepsin. The sequence information in Fig. 3 was used as input to A. McLachlan's comparison program[21] which showed 11 well defined regions of high homology (see also Fig. 4). The two regions of highest homology are those in the neighbourhood of the two active site aspartic acid residues (residues 29–42 and 212–221). The relatively high degree of sequence identity (32%) suggests that the tertiary structures of these two enzymes should be very similar with differences in conformation associated mainly with surface loops as has been

	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	A 15	B 15	16	17	18	19	20	21	22	23	24
Pen.	A	A	S	G	V	A	T	N	T	P	T	A	N	-	D	E	E	Y	I	T	P	V	T	I	G	-	-	-	-	G
Pep.	A	A	A	L	I	G	D	E	P	L	E	N	Y	L	D	T	E	Y	F	-	-	G	T	I	G	I	G	T	P	A

	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Pen.	T	T	L	N	L	N	F	D	T	G	S	A	D	L	W	V	F	S	T	E	L	P	A	S	Q	Q	S	G	H	S
Pep.	Q	D	F	T	V	I	F	D	T	G	S	S	N	L	W	V	P	S	V	Y	C	S	S	L	A	C	S	D	H	N

	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	A 80	81	82	83
Pen.	V	Y	N	P	S	A	T	G	K	-	E	L	S	G	Y	T	W	S	I	S	Y	G	D	G	S	S	A	S	G	N
Pep.	Q	F	N	P	D	D	S	S	T	F	E	A	T	S	Q	E	L	S	I	T	Y	G	T	G	S	M	-	T	G	I

	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	1 00	01	02	03	04	05	06	07	08	09	10	11	12	13
Pen.	V	F	T	D	S	V	T	V	G	G	V	T	A	N	G	Q	A	V	Q	A	A	Q	Q	I	S	A	Q	F	Q	Q
Pep.	L	G	Y	D	T	V	Q	V	G	G	I	S	D	T	N	Q	I	F	G	L	S	E	T	E	P	G	S	F	L	Y

	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	A 34	35	36	37	38	39	40	41	42
Pen.	D	T	N	N	D	G	L	L	G	L	A	F	S	S	I	N	T	V	Q	P	Q	S	Q	T	T	F	F	D	T	V
Pep.	Y	A	P	F	D	G	L	L	G	L	A	Y	P	S	I	S	A	S	G	A	T	-	P	V	F	D	N	L	W	D

	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
Pen.	K	S	S	L	A	Q	P	L	F	A	V	A	L	K	H	Q	-	-	Q	P	G	V	Y	D	F	G	F	I	D	S
Pep.	Q	G	L	V	S	Q	D	L	F	S	V	Y	L	S	S	N	D	D	S	G	S	V	V	L	L	G	G	I	D	S

	73	74	75	76	77	78	79	80	81	82	83	84	A 84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	2 00	01
Pen.	S	K	Y	T	G	S	L	T	Y	T	G	V	D	N	S	Q	G	F	W	S	F	N	V	D	S	Y	T	A	G	S
Pep.	S	Y	Y	T	G	S	L	N	W	V	P	V	-	S	V	E	G	Y	W	Q	I	T	L	D	S	I	T	M	D	G

	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Pen.	Q	S	G	D	G	F	-	-	-	S	G	I	A	D	T	G	T	T	L	L	L	L	B	D	S	V	V	S	Q	Y
Pep.	E	T	I	A	C	S	G	G	C	Q	A	I	V	D	T	G	T	S	L	L	T	G	P	T	S	A	I	A	I	N

	32	33	34	35	36	37	38	A 38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Pen.	Y	S	Q	V	S	G	A	Q	Q	D	S	N	A	G	G	Y	V	F	(T	C	S	B	V)	T	B	L	P	V	S	I
Pep.	I	Q	S	D	I	G	A	-	S	E	N	S	D	G	E	M	V	I	S	C	S	S	I	D	S	L	P	D	I	V

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	A 82	83	84	85	86	87	88	89
Pen.	S	G	Y	-	T	A	T	V	P	G	S	L	I	N	Y	G	P	S	G	N	G	S	T	C	L	G	G	I	Q	S
Pep.	F	T	I	D	G	V	Q	Y	P	L	S	P	S	A	Y	I	L	Q	D	D	D	S	-	C	T	S	G	F	E	G

	90	91	92	93	94	95	96	97	98	99	3 00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19
Pen.	N	-	-	-	-	S	G	I	G	Y	(L	I	L)	G	D	I	F	L	K	S	Q	Y	V	V	F	D	S	D	G	P
Pep.	M	D	V	P	T	S	S	G	E	L	W	I	L	G	D	V	F	I	R	Q	Y	Y	T	V	F	D	R	A	N	N

	20	21	22	23	24	25	26	27
Pen.	Q	L	G	F	A	P	Q	A
Pep.	K	V	G	L	A	P	V	A

Fig. 3 Comparison of tentative penicillopepsin (Pen) sequence with porcine pepsin (Pep). Amino acid residues enclosed by solid lines are those regions of highest homology when compared with McLachlan's[21] sequence comparison program. Tentative sequence identity with pig pepsin: 102 residues = 31.7%. The single letter code for the amino acids is given in ref. 46. For penicillopepsin, the order of the fragments from chemical sequencing[9] with the residue numbers in this table is as follows: N-terminal fragment (−4 to 111); PP3 (111 to 121); PP7 (122 to 125); PP1 (126 to 138); PP2 (139 to 150); central fragment (151 to 197); PP5 (198 to 207); DAN (211 to 220); PP4 (221 to 231); PP10 (232 to 246); PP11 (247 to 259); PP6 (260 to 263); PP12 (265 to 299); X-ray map (300 to 302); C-terminal fragment (303 to 327). Fragments PP8 and PP9 (nine residues) have not been used in this sequence. There is a major discrepancy between the chemical sequence of PP11 and the electron density map. Sequence numbering is that of porcine pepsin[5]. The identification of residues enclosed in parentheses comes mainly from the electron density and not from chemical sequencing and therefore these regions are tentative.

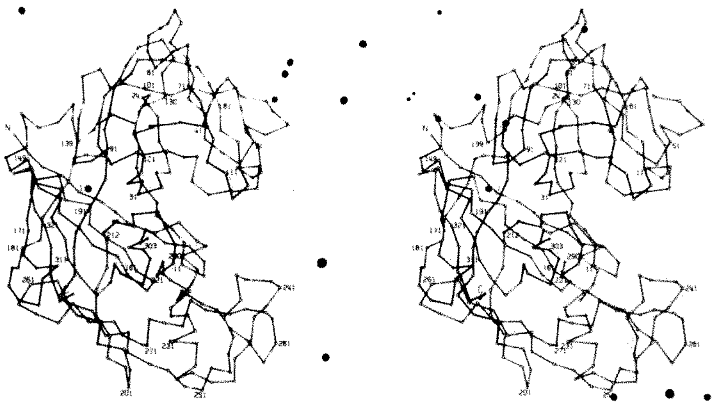


Fig. 4 Stereo-drawing of the main polypeptide chain in penicillopepsin. The circles represent α-carbon positions and only every tenth residue has been numbered (corresponding to the numbering of pig pepsin[5]). The regions of high amino-acid sequence homology (see Fig. 3) are drawn in dark lines. The bilobal feature of the molecule is evident; the active site is located in the groove that separates the two lobes.

observed in comparison of the bacterial serine proteases with the pancreatic enzymes[15,16,22,23].

Polypeptide chain conformation

The folding of the polypeptide chain of penicillopepsin is shown diagrammatically in Fig. 4. The overall dimensions of the molecule are approximately 65 × 49 × 39 Å and this asymmetric shape agrees with the dimensions reported for the low resolution studies of two other acid proteases[7,8]. The molecule is distinctly bilobal with an extended hydrophobic interior in the shape of a croissant. The two lobes are separated by a deep cleft which is approximately perpendicular to the 65-Å molecular length. Buried in the interior of this groove, but accessible to solvent, are the two catalytically important residues Asp-32 and Asp-215. The larger of the two lobes (upper part of Fig. 4) is made up of residues −4 to 171; the smaller lobe is composed of the C-terminal part of the chain.

The largest mixed β-sheet structure observed in globular protein structures is found in penicillopepsin and it consists of 18 strands of polypeptide chain. Figure 5 shows this sheet structure diagrammatically and we have described it according to the convention proposed by Richardson[24]. Not only is this the largest β-sheet observed in globular proteins but it also contains two cross-overs (±5X) that have not been observed previously[24]. This sheet starts at one end of the molecule (strand e is located in the uppermost portion of Fig. 4) and twists in the usual anti-clockwise fashion by 540° before reaching the final strand o. The central 10 strands are all anti-parallel, and the strands containing the active site aspartic acid residues (strands c and m) both have neighbouring parallel strands which are partly buried in the hydrophobic interior of the molecule. The topology of this sheet produces the croissant-shaped hydrophobic interior and there are 12 'hairpin' turns that are all located on the enzyme's surface.

Those stretches of polypeptide chain which adopt a helical conformation are 58–62, 137–141, 225–235 and 303-309. The single disulphide bridge in the molecule, Cys-250 to Cys-283 is located on the extremity of the smaller lobe (bottom right of Fig. 4). It is curious that this feature which seems to contribute little towards the overall stability of the structure is so highly conserved in acid proteases[5,9,25]. Porcine pepsin has two additional disulphide bridges, 45–50 and 206–210. The corresponding residues of penicillopepsin, although not cysteines, are sufficiently close to be connected by a disulphide bridge.

Catalytic site

Chemical studies of acid proteases, and the pH dependence of the catalytic rate constants of substrate hydrolysis implicate two carboxyl groups in the catalytic event; one in the protonated form, and the other in the unprotonated form[26]. The inactivation of porcine pepsin and penicillopepsin by diazoacetylphenylalanine ethyl ester[27] and diazoacetylnorleucine ethyl ester[11] (DAN) identified Asp-215 (Fig. 3) as one of the catalytic residues. The inactivation of porcine pepsin by 1,2-epoxy-3(p-nitrophenoxy)propane (EPNP) showed that Asp-32 was probably the other catalytic residue[28]. EPNP also inactivates penicillopepsin[29], although it has not been possible to isolate the labelled peptide. We assume that the site of reaction with EPNP is the same as that in pig pepsin because the electron density map (Fig. 2) shows clearly that the two carboxyl groups of Asp-32 and Asp-215 are in intimate contact. In addition, Asp-32 is in a sequence of five amino acids that are identical in the seven acid proteases examined[30].

Asp-32 is in strand c (Fig. 5 and legend) which is anti-parallel to neighbouring strand m which contains Asp-215. Both of these strands have neighbouring parallel strands h and p, respectively. Figure 3 shows the high degree of sequence homology for these two pairs of strands and the conformation of the chain in these pairs is very similar; both strand c and strand m have a sharp bend at the two glycine residues 34 and 217. There does not seem to be main chain hydrogen bonding between strands c and m, however there is clearly hydrogen bonding of Thr-33 side chain to the main chain between Ala-214 and Asp-215 and a corresponding interaction of the side chain of Thr-216 with main chain between Phe-31 and Asp-32. Both strands h and p are internal strands, which is usually the case for parallel β structure[24].

Maleic acid has two carboxyl groups which interact[31] and the pK$_a$ values of these groups are 1.83 and 6.07

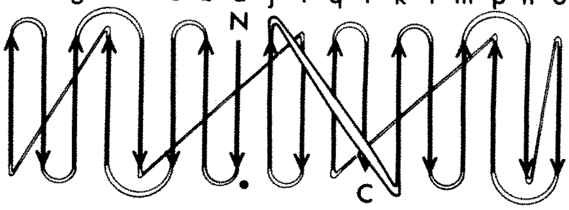


Fig. 5 Diagrammatic representation of the 18-stranded mixed β-pleated sheet structure. This diagram does not display surface topology which can be seen in Fig. 4. Strands of polypeptide chain are labelled a–r from the N to C terminus. The residue numbers at the start and end of each strand are: a (−2 → 8), b (11 → 18), c (25 → 33), d (36 → 41), e (62 → 66), f (84 → 87), g (98 → 104), h (118 → 124), i (150 → 156), j (161 → 168), k (179 → 186), l (189 → 199), m (202 → 216), n (218 → 224), o (286 → 295), p (298 → 303), q (309 → 316), r (319 → 325). The sequence of connection types according to the convention of Richardson[24] is −1, −1, −2, −3X, +1, +2, +5X, −1, +4X, +1, +1, +2, +1X, −2, −5X, +1.

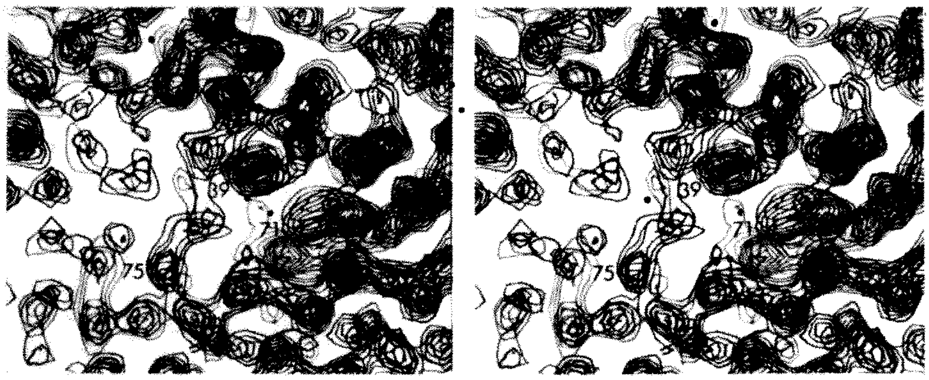
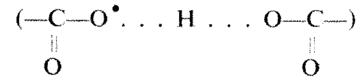


Fig. 6 Seven sections (1-Å spacing) of the electron density map in the region of Trp-39, Trp-71 and Tyr-75. The stacking of the rings of Trp-39 and Trp-71 is shown and the edge of Trp-39 is in contact with the solvent channel of the substrate-binding groove.

(ref. 32). Both maleic acid and the maleate monoanion[33,34] have a proton shared between two oxygen atoms



$$(\text{—}\underset{\underset{\text{O}}{\|}}{\text{C}}\text{—O}^{\bullet}\ldots\text{H}\ldots\text{O—}\underset{\underset{\text{O}}{\|}}{\text{C}}\text{—})$$

The interaction of the carboxyl groups of Asp-32 and Asp-215 probably occurs through a similar sharing of a proton. The proximity of these groups could satisfy the requirement for a protonated and an unprotonated group[26]. The pH-dependence of the DNA-reaction with Asp-215 (refs 27, 35) and the EPNP-reaction with Asp-32 (refs 29, 36), suggests that in the conditions of the modification reactions, Asp-215 has a higher pK_a ($\sim$4.5) than that of Asp-32 ($\sim$1.5). The two carboxyl groups as seen in the present crystal structure are not necessarily in the same ionisation state because there are neighbouring side and main chains which interact with the carboxyl groups in a non-symmetric manner. Various hydrogen bonding interactions occur with the Asp-32 carboxyl group which is the first carboxyl group to be deprotonated on raising the pH above 1. The oxygen, of the carboxylate group, which is hydrogen bonded to Asp-215 also interacts with main chain between Thr-216 and Gly-217; the internally buried carboxyl group of Asp-304 interacts with the opposite side of this peptide bond (see Fig. 2). Ser-35 is hydrogen bonded to the other carboxylate oxygen of Asp-32, either directly or through a solvent bridge. There are no equivalent interactions seen for Asp-215 although the side chain of Thr-218 is nearby. In addition, there is an ion-pair (Lys-308 with Asp-11) in the vicinity of Asp-304 and a conformational change brought about by rotation of the carboxyl group of Asp-304 around its $C\alpha$–$C\beta$ bond could change the ion-pair to one between Lys-308 and Asp-304. The positive charge of Lys-308 in penicillopepsin is conserved in the mammalian enzymes by having an arginine residue at this position in pepsin and chymosin[37]. The implications of these interactions which involve conserved residues in all the acid protease sequences thus far determined[30,37] must await detailed model building of penicillopepsin and a comparison with other high resolution protease structures.

Binding groove

Although we have no direct evidence in the form of difference electron density maps of inhibitor or substrate analog binding, we postulate that the deep groove which separates the two lobes of the molecule is the extended substrate binding site common to acid proteases[10,26,38]. There are three reasons for this proposal. (1) The two active site aspartic acid residues are located inside the groove and towards one end of it. (2) There is a cluster of three aromatic residues which are very close to the surface of the groove and are located in the larger lobe at distances between 8 and 13 Å from the active site. Trp-39 and Trp-71 have their ring systems stacked and one edge of Trp-39 is in contact with the solvent channel of the groove (see Fig. 6) Tyrosine-75 is also located in this region and is in contact with solvent. Chemical studies with the peptide Leu-Gly-Leu have shown that it binds to penicillopepsin, activates the hydrolysis of Leu–Tyr–NH$_2$, inhibits the penicillopepsin activation of trypsinogen and produces changes in three of the transition bands (290, 286 and 276 nm) of the CD spectrum of penicillopepsin[39]. We suggest that the binding of Leu–Gly–Leu and other activator peptides[10] in the groove perturbs Trp-39, Trp-71 (the bands at 290 and 286 nm) and Tyr-75 (276 nm) and that this perturbation on binding causes the circular dichroism spectral changes. There is only one other tryptophan residue, Trp-190, and its circular dichroism band (293 nm) does not change when activator peptides are bound to penicillopepsin. Trp-190 is buried in the hydrophobic core, inaccessible to solvent and removed from the active site region. (3) The deep groove observed in the low-resolution study of the acid protease from *R. chinensis*[7] is the site of binding of inhibitors to this enzyme. As the other acid proteases have a cleft in the molecule, it is probable that the feature we observe is the extended substrate binding cleft.

Enzymatic studies have led to estimates of the total length of the substrate binding groove of seven[40] to eight or nine amino acid residues[41]. For a fully extended substrate chain this would correspond to a length of 26–32 Å. In penicillopepsin we have estimated the length of the binding site on the N-terminal side of the scissile bond (secondary site A and primary site S$_1$ as defined by Takahashi and Hofmann[42]) and found it to be equivalent in length to a tetra or pentapeptide[43] (14–18 Å). From the length of the groove estimated on the model ($\sim$30 Å) and the circular dichroism evidence, we conclude that Leu–Gly–Leu binds in the secondary site A (ref. 43) and that this site is located in the deeper and longer part of the groove (to the right of the catalytic site in Fig. 4).

Comparison with other acid proteases

The amino acid sequence comparison in Fig. 3 shows regions of extensive homology between penicillopepsin and porcine pepsin. The overall shape and dimensions of penicillopepsin are very similar to those of the acid proteases of *R. chinensis*[7] and *E. parasitica*[8]. Insufficient information is available for a detailed comparison with the 2.7-Å structure of pig pepsin[6], but the reported tracing of the path of the polypeptide chain of pepsin[6] shows no homology to the structure we report here. Although Fruton[10] has suggested that pepsin and carboxypeptidase A have similar enzymatic mechanisms, the tertiary structure of penicillopepsin is distinctly different to that of carboxypeptidase A, which has an extensive arrangement of 8 strands in mixed parallel and anti-parallel β-sheet conformation[44]. We have compared, visually, the polypeptide chain folding of penicillopepsin reported here to those of the acid proteases from *R. chinensis* and *E. parasitica*. The three structures are in close agreement, strengthening the argument that the porcine pepsin structure will be found to be similar.

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letters to nature

A conspicuous globule in the Southern Coalsack

THE Southern Coalsack is the finest dark nebula in the Southern Milky Way. At the Royal Observatory, Edinburgh, in October 1976, we studied two photographs taken with the SRC 1.2-m Schmidt telescope (a Unit of the Royal Observatory, Edinburgh) at Siding Spring Observatory, Australia. Centred at α (1975) = 12h 45min, δ (1975) = −63°01′, they show the whole of the Coalsack in all its beauty. The blue plate (Fig. 1) was a 60-min exposure on a hydrogen-sensitised IIIaJ plate, taken through a 2-mm Schott GG395 filter; the near-infrared plate was a 60-min exposure on a water-hypersensitised IN plate, taken through a 4-mm RG715 filter. These plates were taken as part of a more comprehensive study of the Southern Coalsack; this note represents a pilot study of how much can be learned by simple star-counting techniques. Visual inspection of these plates confirms the earlier conclusions[1–3] that the Southern Coalsack is basically a thin obscuring sheet with an average total visual absorption $A_v = 1.2$ mags. Rodgers[1] derived the distance from the Sun to the Coalsack to be 175 pc.

A globule is a roundish, sharply bounded dark nebula with an angular diameter of a few minutes of arc, seen projected as a 'Loch im Himmel' (William Herschel) against a star-rich background. The present pair of Schmidt photographs show a large number of potential globules. A general catalogue is being compiled of globules identified on SRC Schmidt plates of the Southern Milky Way. (This search is being undertaken at Siding Spring by Drs W. M. Goss, R. Manchester, T. G. Hawarden, A. J. Longmore and I. J. Danziger.)

Westerlund was the first to draw attention to the numerous globules in the Southern Coalsack. His original conclusions have been confirmed and extended by Tapia[4]. The most obscured (star-poor) regions in Tapia's Survey are the regions Tapia I and Tapia III. In our survey we have catalogued 27 globules and patches of dense obscuration associated with the Coalsack, some of which are identified in Fig. 1. The first six objects in our catalogue are the globules listed in Table III of Tapia's paper. The densest and most nearly perfect of these globules is the second one in Tapia's list, located at α(1975) = 12 h 30.0 min, δ(1975) = 63°37′ in the region Tapia III. It has an angular diameter of about 6 arc min (0.3 pc). We will refer to this as globule 2.

A comprehensive study of these globules awaits *inter alia* the establishment of standard magnitude sequences in B, V, R and I, to $V = 22$. Useful information on globule 2 has been obtained, however, from inspection of the two available plates. At the suggestion of Dr R. D. Cannon, and with the assistance of Mr R. D. Eberst (both of the Royal Observatory, Edinburgh) a modified Zeiss Blink Comparator has been used to make a fairly complete study of the optical properties of globule 2 by applying simple star-counting methods. The comparator has been modified by J. M. Allen to incorporate a closed circuit TV system similar to that designed and built by J. G. Bolton at ANRAO, Parkes, N.S.W. In addition to the blink facility, the images on a pair of plates may be viewed on the TV monitor simultaneously or separately, side by side or superimposed, or in split-screen modes, by using the appropriate 'wipe' or 'fader' facilities. In the present case the infrared and the blue plates were inspected in a nearly superimposed mode, with the infrared images appearing as black dots and the blue images as white dots in the TV display (Fig. 2).

Beyond the outer limits of globule 2 each star shows both red and blue images, generally right up to the rather sharp outer edge of the globule at about 3 arc min from its centre, measured on the blue (J) plate. In the annulus lying between 3 and 1.6 arc min from the centre of the globule, 37 stars have red images without blue companions, and only four stars in this annulus have faint blue images. There are no stars visible in either colour within 1.6 arc min of the globule centre. The star counts were done from polaroid photographs of the TV display. These simple observations show that the dust in globule 2 is highly concentrated towards its centre. Similar counts were made in a comparison field in a star-rich region 17 arc min south of globule 2. In this field 186 stars had images on the infrared plate, and of these 170 stars also appeared on the blue plate, in an annulus of radii 1.6 and 3 arc min.

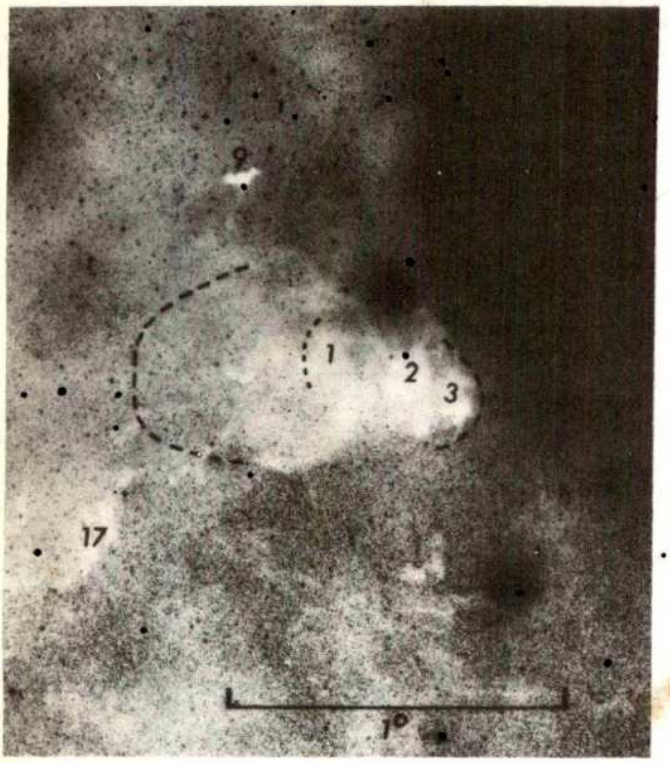

Fig. 1 Part of the Southern Coalsack, reproduced from a deep IIIaJ plate taken with the U.K. 1.2-m Schmidt telescope at Siding Spring. Globules 1, 2 and 3 in Tapia's list are numbered. α Crucis is shown on the right (west) of the print. The long-dashed curve indicates the approximate boundary of the HI absorption observed by Kerr *et al.*[8], and the short-dashed curve shows the eastern boundary of the formaldehyde absorption observed by Brooks *et al.*[9].

An approximate minimum dust mass for globule 2 can easily be derived from the star counts using the methods described by Bok, Cordwell and Cromwell[5] and in the doctoral thesis of Dickman[6]. We base our calculations on the log $A(m)$ against m curves in the blue and near infrared shown by Tapia[4]. These curves were derived from stars between the 12th and 16th magnitude, but the limiting magnitude of the blue Schmidt plate is at least B]22. Star-count data to 21.0 mag (ref. 7) indicate that the slopes to be used in our calculations should be approximately two-thirds of those derived by Tapia. Comparisons of the near-infrared star counts in the annulus round the globule 2 and in a comparable area in the comparison field yield a value of the average absorption in the near infrared (about 8,500 Å) $A_I = 2.7$ mag. Corresponding values for A_B and A_V are $A_B = 6.5$ mag, $A_V = 5.0$ mag. The value of A_B is roughly confirmed by the presence of four stars in the annulus which have faint blue images. This is about the number we would expect for $A_B = 6.6$ mag. The values quoted apply strictly only to the annulus between 1.6 and 3 arc min from the centre of the globule.

A minimum dust mass for the entire globule can be obtained by applying the above values to the whole of the globule. The mass thus derived underestimates the total contribution from the core, for which we know only that A_V and A_B must be greater than the values given above for the outer ring. The derived minimum dust mass is $0.05M_\odot$. Using a factor 200 to proceed from the dust mass to the total mass of dust and gas (presumably mostly molecular hydrogen, H_2), we derive an approximate minimum mass of $10M_\odot$ for globule 2. This is a minimal value since we have not been able to penetrate the 1.6-arc min diameter core of this globule, which may be quite massive, possibly as high as $25M_\odot$. The estimate of the minimum total mass should be halved, however, if the dust to gas ratio in this region is assumed to be 1 : 100. We note that the total absorption A_V enters linearly into the formulae from which the total mass is derived.

Kerr, Hawarden and Bowers[8] searched for HI self-absorption in 19 regions in the Coalsack, and found it only in the region of globule 1, 2 and 3. The neutral hydrogen peaks over globules 1 and 2 and tails off for 35 arc min east of globule 1. In the outer edges of the HI distribution the optical absorption is much lower than in other regions where no HI absorption was seen. Brooks, Sinclair and Manefield[9] have observed the distribution of OH 1,667-MHz emission and formaldehyde 4,830-MHz absorption over the complex. The OH distribution resembles the HI distribution (and hence the optical absorption distribution shown in Fig. 1) while the formaldehyde was detected only in the regions of highest dust density, outside of which it falls off steeply on all sides. Sinclair and Brooks[10] estimated the diameter of the formaldehyde cloud in globule 2 to be 8 arc min, very similar to the optical diameter found from our photographs of globule 2 and its close neighbours.

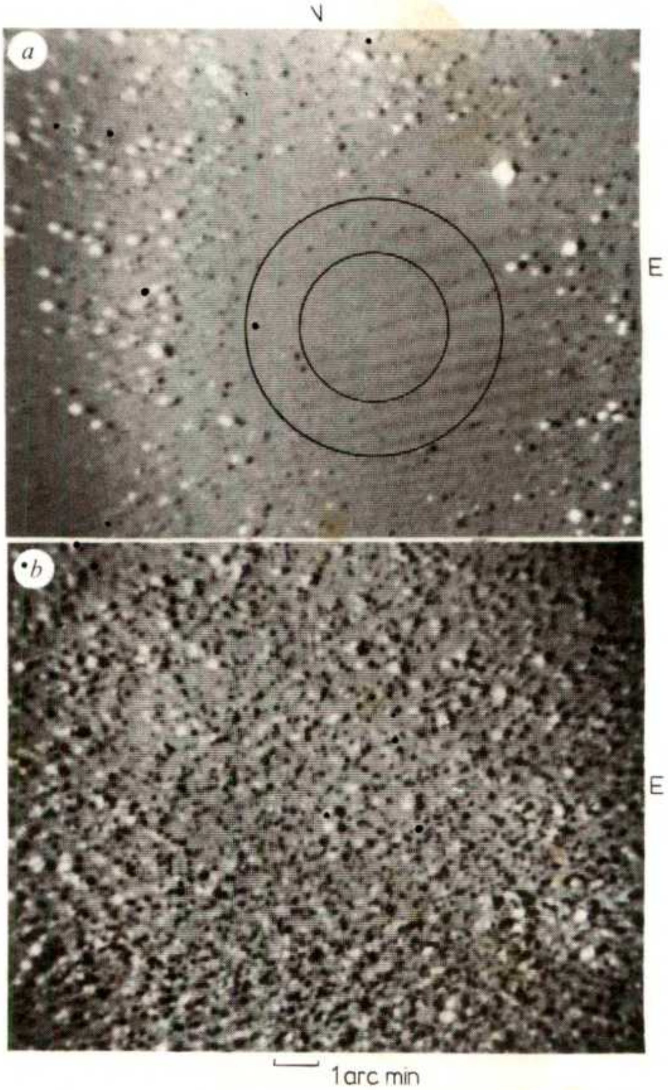

Fig. 2 TV monitor display of *a* globule 2, and *b* the comparison field. Images from the blue plate are shown in white; images from the near infrared plate are shown in black.

Radio observations are beginning to yield much additional information on the physical conditions in the complex around globule 2. A team from Queen Mary College, London[11], have used the 3.9-m Anglo–Australian telescope to observe the 2.6-mm line of CO at six points. Their preliminary results show that the CO emission also peaks over globule 2, for which they derive a mass of several solar masses and kinetic temperatures between 6 and 16 K. Dickman's results for globules accessible from northern latitudes would suggest probable numbers of ^{13}CO molecules in globule 2 of between $N_{13} = 10^{16}$ and $N_{13} = 2 \times 10^{16}$. His formula for the mass of the molecular hydrogen in g cm^{-2} is: N_{mass} (g cm^{-2})

$= 2 \times 10^{-18} \times N_{13}$. From the widths of the lines in the radio spectrum it should also be possible to derive the velocity dispersion of the CO molecules and any rotation of the globule, if present, should be detectable to within 0.1–0.2 km s^{-1}.

The studies of globules by Martin and Barrett[12] and by Tucker *et al.*[13] show that globules are most likely to be gravitationally bound and in a state of collapse. The smaller ones among the large globules, such as Barnard 335, have radii of the order of 0.3 pc and masses of about 25 $M_\odot$. Larger objects in this class, such as Barnard 5, have radii of the order of 1.2 pc and masses as high as 800 $M_\odot$. Globule 2 ranks as being smaller and a little less massive than Barnard 335. The smaller ones will probably collapse into single or more likely multiple stars, whereas those of higher masses may collapse into modest star clusters. Globule 2, as well as most other globules in the Southern Coalsack, is probably on the verge of collapsing into a single or multiple star. The time required for collapse into a protostar (or a modest protocluster) is probably < 1 Myr.

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Periodic fadings in VHF radio-satellite transmissions during the solar eclipse on 23 October 1976

THE localised time-dependent cooling action of the lunar shadow during an eclipse may generate gravity waves at ionospheric heights[1,2]. The bow wave thus formed would be detected at great distances from the eclipse path. Experimental data in the northern hemisphere from the eclipse on 7 March 1970, indicated the presence of gravity waves in the F-region, deduced from the incoherent backscatter technique[3], vertical incidence soundings[4], and recordings of the Faraday rotation angle of VHF transmissions from geostationary satellites[5]. The detected travelling ionospheric disturbance (TID) had the horizontal phase velocity of 400 m s^{-1} (ref. 4) and 620 ± 120 m s^{-1} (ref. 5). TID was reported to travel north to south[4], or 279 ± 25° east of north[5]. We report here our observations of large-magnitude periodic fadings during the eclipse of 23 October 1976, which indicate that the eclipse is associated with generation of wave disturbance affecting radio-satellite transmissions in the upper VHF. The waves had a 40-km wavelength, and propagated from north to south with a velocity of 556 m s^{-1}.

It seems that no experiment has been conducted on the effect of the solar eclipse on the amplitude of radio transmissions from non-synchronous satellites. This was mainly due to the limited number of orbiting satellites used in previous scintillation studies. Thus, the probability of coincidence of an eclipse and a satellite pass was small. Our results are based on amplitude recordings of VHF radio signals from six U.S. Navy Navigational Satellites (NNSS) transmitting at a frequency of 149.988 MHz, and D2-B satellite (catalogue No. 8332) transmitting at a frequency of 136.740 MHz. NNSS and D2-B had orbit inclinations of 90 and 37°, respectively. The recording station was at Brisbane (27.5°S, 152.9°E geog. lat. and long., and 37.2°S invar. lat.).

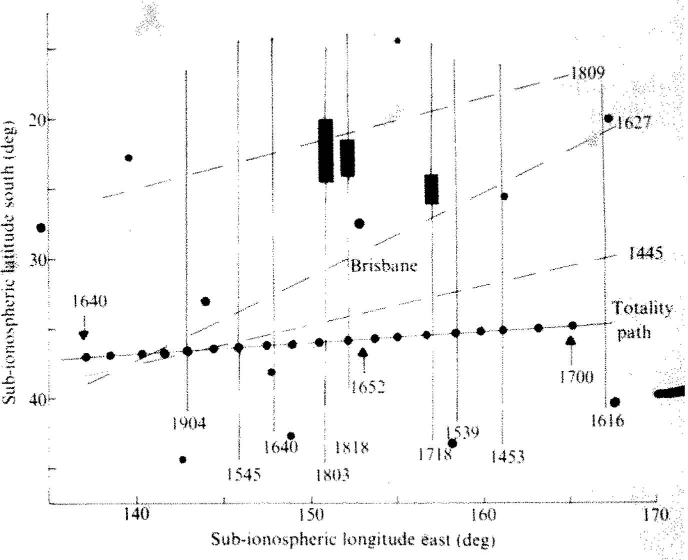

Fig.1 Sub-ionospheric orbits of NNSS (—) and D2-B (– – –) satellites during the solar eclipse on 23 October 1976. The first periodic fadings (vertical bar) were recorded during a NNSS pass at 1718 LT after the time of the closest approach of totality at 1652 LT. Arrows show the central positions of the eclipse at different local times.

The path of eclipse totality on 23 October 1976, ran south of the Australian continent between about 1600 and 1700 LT (150° EST). It was possible to record a relatively large number of satellite passes during the eclipse period, using radio transmissions from the seven orbiting satellites. Figure 1 shows the sub-ionospheric orbits for NNSS and D2-B at 400-km altitude. The satellite passes at 1445, 1453, 1539, 1545, 1616, 1627 and 1640 LT (times of the predicted nearest approaches) did not show any scintillation activity. A NNSS pass at 1718 LT revealed pronounced periodic fluctuations of a large magnitude (3 dB peak-to-peak) superimposed over normal, slow variations in amplitude (Fig. 2). The disturbed region was north of Brisbane (vertical bar in Fig. 1). Assuming that the disturbance occurred at the point of closest approach of totality at 1652 LT, the velocity of propagation of the waves is 556 m s^{-1}.

The following NNSS passes at 1803 and 1818 LT indicated the presence of periodic fadings of an unusually large magnitude (5 dB peak-to-peak in Fig. 2), confined to the region further north of Brisbane (Fig. 1). The periodic fadings were absent during a pass at 1904 LT. A D2-B pass at 1809 LT from south-west to north-east did not reveal any periodic fluctuations (Fig. 1), indicating that the wave-front was directed more towards the geographic north than east–west, since radio fadings are enhanced in the direction of the steep ionisation gradient generated by the passage of a periodic disturbance. It should be also noted that the largest periodic fadings were recorded about the time when the eclipse terminated. This may be associated with the enhancement of TIDs at the end of the eclipse due to an interference effect of the waves generated at various positions of the totality. The possibility of such enhancement was suggested in association with the eclipse on 7 March 1970 (ref. 1).

The disturbances shown in Fig. 2 had a typical period of about 10 s, which corresponds to a 40-km wavelength at ionospheric heights. This is considerably smaller than the wavelengths found from the ionospheric experiments conducted during the 1970 eclipse. For example, the total electron measurements[5] indicated a 20-min period of the disturbance, which corresponds to a wavelength of about 700 km. This difference may be due to the various spectral characteristics of the techniques used for detection of ionospheric fluctuations; the total electron and satellite scintillation methods tend to indicate long and short scale sizes of the disturbance, respectively. From this, it seems that the eclipse generates TIDs with a number of wavelengths ranging from about 40 to 700 km.

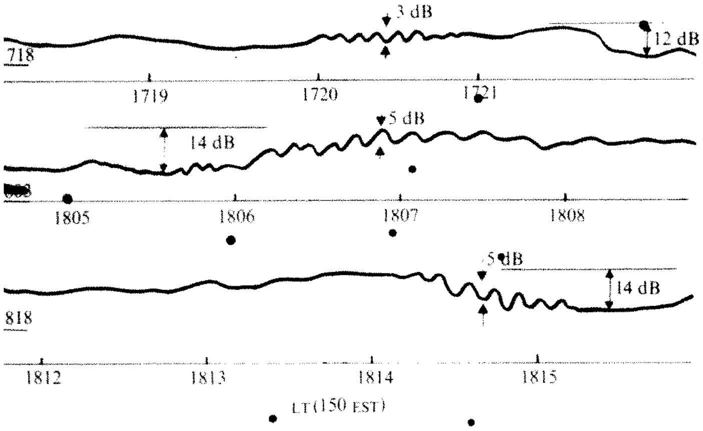

Fig. 2 Amplitude structures of periodic fadings in VHF radio-satellite transmissions during the eclipse period.

The distinct and prolonged periodic fadings (Fig. 2) are an unusual feature of the radio-satellite experiment conducted over several years at Brisbane. Amplitude records obtained from 150 satellite passes in October, 1976, indicated the presence of periodic fadings in 3% of passes. The periodic fadings recorded at other times than the eclipse period had a less well-defined quasi-sinusoidal structure and their magnitude was $\leqslant 3$ dB peak–peak. Also, their duration was limited to one satellite pass only, unlike the events shown in Figs 1 and 2, which were recorded during three consecutive satellite passes.

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Electrons and positive ions in an auroral arc

Two new types of British sounding rocket, a Skylark 12 and a Fulmar, were launched from Andøya in northern Norway, on 21 November 1976, in an experiment to study the particle streams and plasma waves associated with an auroral arc. We report here the first results obtained from channel multipliers and electrostatic analysers carried on the Skylark 12 (designation SL 1422) to detect electrons in the range 0.5–25 keV, and positive ions of 0.5–25 keV per unit charge. We believe that the results are unique and constitute the first detailed comparison of positively- and negatively-charged particles in the region of an auroral arc. They have been made possible by the combined advantages of high altitude (715 km), which enables ions to be studied before absorption in the atmosphere, long range (780 km), which allows a complete traversal of an auroral structure, and new analysers, which extend the dynamic range of measurement.

Spectrograms of positive ions and electrons taken from near the middle of the flight are shown in Fig. 1. In this period the rocket, travelling northwards, left the diffuse aurora to the south of the arc to enter at 265 s the intense stream of electrons that produced the luminous east–west curtain of the visible arc. The period shown also encompasses the rocket's emergence at 360 s into a region of sub-visual aurora to the north of the arc.

We note first that the arc is characterised by relatively high electron intensities and energy spectra with a pronounced peak whose position varies within the arc between 2 and 9 keV. We note also the marked anticorrelation between electron and ion intensities during the transition between the diffuse aurora and the arc. The anticorrelation does not persist far into the arc: between 305 and 330 s, although there is little obvious change in the electron intensity, the ion intensity rises to form a spectrum with a broad peak around 6 keV, a minimum near 2 keV, and a tail rising towards low energies. This ion spectrum is unlike those measured between 150 and 250 s which are approximately Maxwellian with a most probable energy of 10 keV. To the north of the arc after 360 s the most probable energy of the ions is 3 keV, and the electron spectrum has a power-law form with intensities decreasing towards higher energies. (Unfortunately, in the presence of unremoved telemetry noise, only some of these features are readily discernible in the black-and-white spectogram of Fig. 1.)

We offer the following preliminary interpretation of these results based on conclusions drawn from earlier lower-altitude flights about the mechanism for electron acceleration in auroral arcs[1,2]. To the south of the arc we suppose the electrons and ions to be scattered without significant acceleration from their source plasma, the plasma sheet of the magnetosphere, where electron and ion temperatures correspond to 2 and 10 keV, respectively. The particles forming the arc are from the same source, but in this case the electrons are accelerated during precipitation by the equivalent of a potential difference that fluctuates rapidly about a most probable value indicated by the position of the peak in the spectrum. The fluctuations are invoked[1,2] to account for the breadth of the peak on the low-energy side; the breadth on the high-energy side is simply a consequence of the finite temperature of the source plasma. Ions, being subject to the same potential differences, are retarded and reduced in intensity when electrons are accelerated. This would account for the anticorrelation near the southern border of the arc. We suggest that the increase in ion intensities near the centre of the arc, where electrons still seem to be accelerated, is an example of an occurrence predicted earlier[3], namely, that at times the effective potential difference could fluctuate enough to reverse in sign temporarily and apparently accelerate ions and electrons simultaneously. The peak in the ion spectrum, being sharper than that of the Maxwellian spectra to the south, is consistent with acceleration taking place at this time. The facts that the ion peak is broader than the electron peak and the ion intensity is less enhanced than the

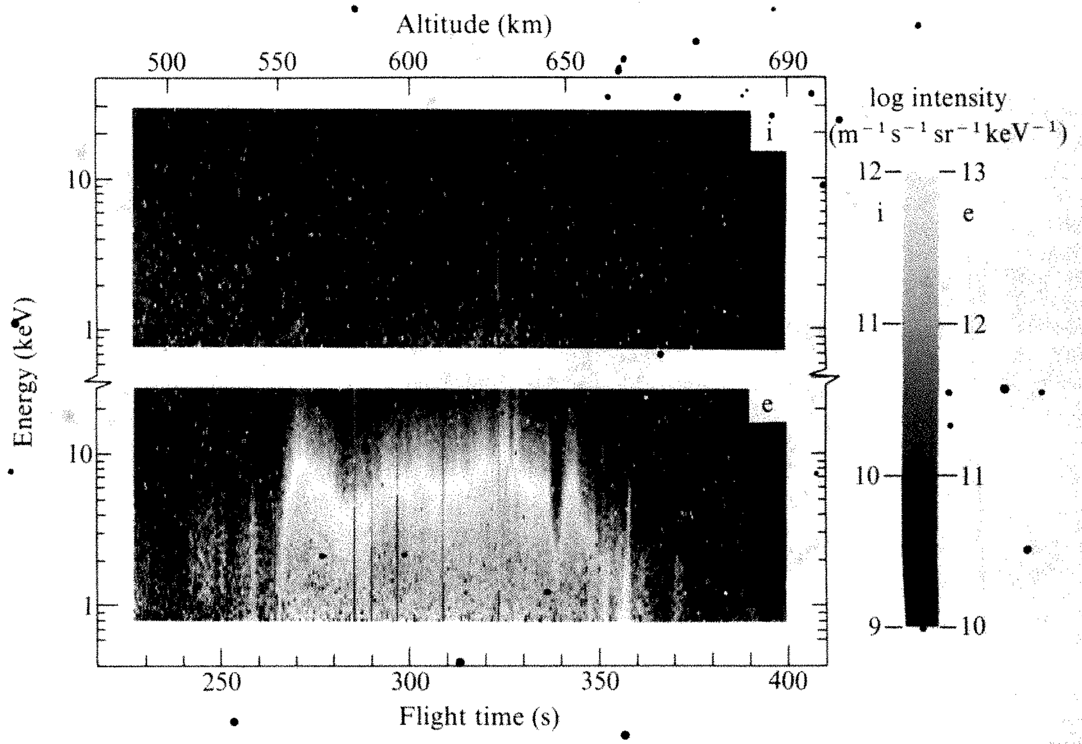

Fig. 1 Positive-ion (i) and electron (e) spectrograms from SL 1422 launched at 2115 UT on 21 November 1976. The intensities were measured at pitch angles in the range 30–40°.

electron intensity indicate a broader distribution of energy gain for the acceleration of positively-charged particles. North of the arc the electrons and ions are of lower energy than those to the south, and the electron spectra are different in form. This supports the suggestion made previously, based on shorter-range flights[4,5], that auroral arcs are phenomena occurring at the boundary between the plasma sheet and high-latitude magnetotail regions of the magnetosphere.

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Speckle image reconstruction of solar features

SPECKLE imaging is a process which permits recovery of diffraction limited resolution from atmospherically degraded astronomical images. We describe here the application of this new process to the reconstruction of solar surface features with resolution approaching the telescope limit, despite severe atmospheric blurring in the original data.

Speckle imaging has evolved from speckle interferometry, which has been used to measure stellar diameters[1] and binary stars[2] to an angular resolution of 0.025 arc s and has yielded evidence of sub-arc s surface detail when applied to solar images[3,4]. In speckle interferometry only image autocorrelations are obtained, restricting information recovery to centrosymmetric detail. In speckle imaging, true diffraction limited images of extended objects can be reconstructed without requirement of a reference star in the field.

The data-taking requirements are identical for both imaging and interferometry: a series of short exposure, narrow pass-band, highly magnified images are recorded on film. For most low light level astronomical objects, an image intensified camera is used, though for solar work no intensifier is required. The conventional procedure for processing speckle interferometry data is to Fourier transform individual images and sum their power spectra. In the imaging process, the phase in the Fourier transform of each frame is encoded in the form of point-to-point phase differences. This is performed in two dimensions by calculating the shifted complex product of the transform. The results for each frame are summed, yielding averaged quantities $\langle A(u,v)\rangle$, in the form

$$\begin{aligned}\langle A_u(u,v)\rangle &= \langle I(u,v)\, I^*(u+\Delta u,v)\rangle \\ \langle A_v(u,v)\rangle &= \langle I(u,v)\, I^*(u,v+\Delta v)\rangle\end{aligned} \qquad (1)$$

where $I(u,v)$ is the image Fourier transform and the two averages encode the phase in the u and v directions, respectively. The shifts, Δu and Δv, must be small compared to the resolution element in the object transform.

Each of the components in equation (1) can be expanded in the form

$$\begin{aligned}\langle A(u,v)\rangle \simeq & |O(u,v)|^2 \exp\{i[\Phi(u,v)-\Phi(u+\Delta u,v)]\}\times \\ & \times\langle |T(u,v)|^2 \exp\{i[\Psi(u,v)-\Psi(u+\Delta u,v)]\}\rangle\end{aligned} \qquad (2)$$

where $O(u,v)$ is the object Fourier transform, $T(u,v)$ is the atmospheric transfer function, and $\Phi(u,v)$ and $\Psi(u,v)$ are the phase in the object transform and the atmospheric transfer function, respectively.

The transfer function term in equation (2) has an expected value whose phase approaches zero (since the atmospheric phase fluctuations are a stochastic, zero mean process) and whose amplitude approaches the diffraction limited transfer function usually obtained in conventional speckle interferometry. For reconstruction, the relative phase at any point in the transform is calculated by summing the phase differences between that point and an arbitrary reference point over a multiplicity of paths, which greatly reduces the effects of noise

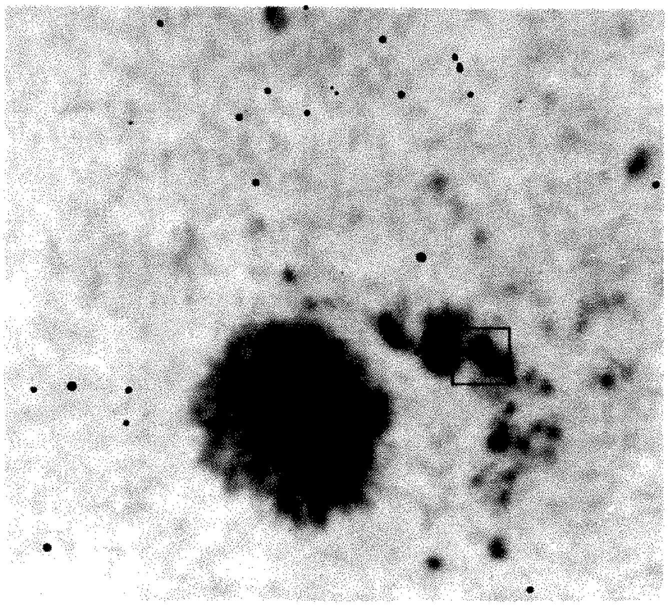


Fig. 1 Photograph of the Sun showing the region processed.

and bad data points. Finally, the output image is obtained by Fourier inversion of the resulting phase and amplitude distribution. This procedure is based on an algorithm suggested by Knox and Thompson[5] and developed and demonstrated for two-dimensional extended, continuous tone objects with simulated atmospheric degradation by Nisenson *et al.*[6]. This process is unrelated to the technique reported by Lynds *et al.*[7], which is applicable only to small objects, close to the telescope diffraction limit in extent. It is also unrelated to the large class of image-processing algorithms in which an inverse impulse response is estimated and applied to restoration of the image.

The photographs processed in this experiment were obtained from Dr J. W. Harvey of Kitt Peak National Observatory and were taken at the 24-inch vacuum solar telescope. The passband was 10 nm centred on 500 nm and the exposure time was $\sim 1/100$ s. At the magnification used, the telescope diffraction limit occurs at a spatial frequency of about 35 lines per mm, well within the transfer characteristics of the Kodak 5460 film. Thirty-two frames were suitable for processing. Figure 1 is a typical frame covering a 2-min square field. Only the indicated 8 × 8-arc-s area, which included a dark feature, was processed. Note the presence of irregular fringe-like interference features in part of the image. These vary randomly from frame to frame, and are coherent phenomena induced by atmospheric turbulence.

On each frame the subarea was scanned and digitised as a 128 × 128 array centred on a selected feature. A 128 × 128 array was chosen to allow two samples per resolution element and to restrict the processed region to an area smaller than the expected isoplanatic patch size. The isoplanatic patch is determined by the angle over which the atmospheric point spread function remains highly correlated.

The digitised frames were then processed as we have described. Each frame was digitally Fourier transformed using an FFT algorithm and, after phase coding, the transforms were added together and the phase reconstruction algorithm applied. The phase and amplitude distributions derived from the averaging process are then retransformed to obtain an image.

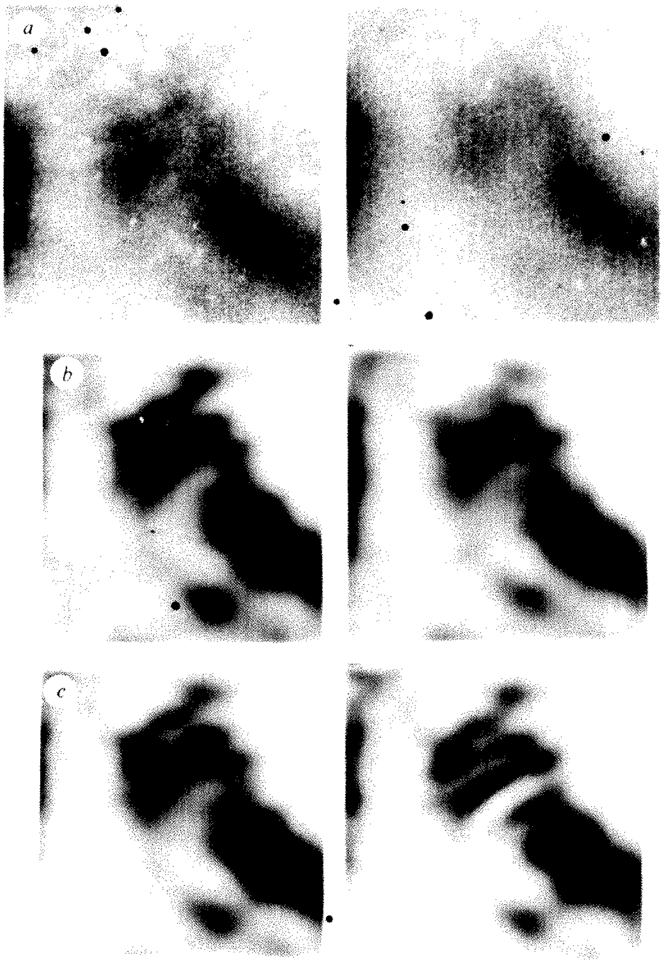
Figure 2 includes two typical input frames after scanning and redisplay. The seeing limit varies from frame to frame and from region to region in the full field (see Fig. 1); however, the average is about 2 arc s, which is 12 times the telescope diffraction limit.

Separate reconstructions produced from 4, 8, 12, 16, 24 and 32 frames show development of small features with progressively increasing sharpness. Reconstructions produced from sequences of frames selected to be free of defects were also nearly identical to those obtained without selection. Figure 2*b* shows two independent 12-frame reconstructions, demonstrating that the essential features are real. Figure 2*c* shows 24-frame and 32-frame reconstructions. The resolution in the final processed image is better than 0.4 arc s and some features such as the bright lanes in the filamentary structure near the centre of the image may be closer to 0.2 arc s, the telescope limit. The resolution (0.4 arc s) is comparable with that obtained in the Stratoscope balloon experiments.

Processing a smaller subarea may have the effect of increasing the rate of convergence to a diffraction limited image if isoplanatism is a problem. The size of the isoplanatic region for solar observations can only be estimated, and a processed area as large as 8 × 8 arc s may not quite converge to the diffraction limit with only 32 frames.

The processed subarea contains a feature which, from the large scale photograph, would be described as a medium-sized pore. In the reconstruction, the region near the centre of the frame breaks up into a number of dark, parallel, elongated features reminiscent of penumbral structure, while the region below it retains the appearance of a typical pore. The penumbra-like structure is approximately radial to a large nearby spot although it is not connected to it. At least two features looking like very small pores approximately 0.7 and 1.0 arc s in size are detached from the primary structure except for a dark lane. Other dark lanes are evident in the picture, the longest extending about 3 arc s.

Fig. 2 *a*, Two typical unprocessed frames (8 × 8 arc s) used in producing the reconstructions after scanning and redisplay; *b*, two reconstructions produced from independent 12-frame subsets of the data; *c*, 24-frame (left) and 32-frame (right) reconstructions. Resolution is approximately 2.0 arc s in the unprocessed frames and approximately 0.4 arc s in the processed frames.



The lighter regions of the reconstruction show evidence of photospheric granulation. This is particularly interesting because there is virtually no evidence of this structure in the original digitised frames, partly because of the limited dynamic range of the scanning instrument, which was set to scan optimally the spot region. The original negatives of the reconstructions show that the entire photospheric region is covered by granules, the smallest of which are less than 0.5 arc s in diameter.

To obtain wide field reconstructions, individual isoplanatic regions must be processed independently and combined in a mosaic. This is a lengthy process that requires large amounts of computer and scanner time. To solve this problem, an optical processor that performs an analog of the digital process is now being developed[8]. Optical processing allows encoding of the phase and direct summation of the individual transforms. The output of the processor is a single frame that is scanned and digitised for computer reconstruction of the final image. With this system, a number of contiguous isoplanatic regions can be processed in parallel, yielding images of relatively large areas at once.

In conclusion, we would like to point out that the ultimate resolution of the process is the diffraction limit of the telescope, independent of the actual seeing conditions. Data taken on the 60-inch solar telescope at Kitt Peak, for example, could display features down to 1/15 arc s, and this can be extrapolated to even larger apertures.

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Long term solar activity forecasting using high-resolution time spectral analysis

SOLAR activity as measured by sunspot number has been carefully observed since 1749. It seems to have an 11-yr cycle which has been identified as a 22-yr magnetic period with every other 11-yr cycle having a reversed average solar magnetic field. As the amplitude and frequency of the cycle varies slowly with time it has become a point of interest to determine if sunspot activity variation is regular and constant over long periods of time. If it is constant, then it should be possible to make predictions based on historical observations. I show here that the monthly sunspot record (1749–1975) is represented by a Fourier Series, using the spectral analysis method of A. Paul. By evaluating the series, a forecast indicates that the next solar cycle began in December 1976, and will peak above 100 near 1982.

Eddy[1] has pointed out that solar activity may have been greatly different during the Maunder Minimum (a period of very low sunspot activity between 1645 and 1695) than has been generally assumed. For example, Schove[2] relied upon the assumption of nine cycles per century to identify solar maxima back to the fifth century BC. Eddy discounted Schove's estimates of the solar maxima magnitudes during the 1600s as systematically high, noting that the magnitudes were sometimes based on "auroral counts and other unspecified data". Eddy stated "the present cycle of activity may be unusual if not transitory".

On the other hand, Wolff[3] discussed physical processes within the Sun from which it is reasonable to expect long-term persistence of discrete line spectra in the sunspot activity record. He has developed a rigid and differential rotation model whose modes have beat frequencies which are mathematically related to each other. Using only two adjustable frequencies in the model, he predicted 20 of the 25 periodicities observed in the sunspot data by Cole[4] and Currie[5]. The longest period found by Wolff is 178 yr. He considered the difference between the value of 178.0 yr and the 190-yr period found by Cole[4] to be insignificant since the uncertainty[3] in an estimate of a frequency present in a record of length T is commonly given as $\pm 1/(4T)$. Cohen and Lintz[6] have used a 179-yr periodicity, with several other periodicities, to produce a prediction of solar activity to the year 2050. (Of course, an extrapolation of their model backwards in time would not produce the Maunder Minimum, particularly since they use a d.c. component of 47.4 smoothed sunspots in their model.)

Several methods for analysing spectra have been applied to the sunspot record. Cole used the fast Fourier transform (FFT) to analyse the autocorrelation function and the solar cycle phase data of Schove and obtained the power spectral density as a smooth continuous function of frequency. Both Cohen and Lintz, as well as Currie, used the maximum entropy method (MEM) to find the smooth continuous power density function. The FFT and MEM methods estimate the frequency of a suspected spectral line at the point of peak power.

I re-examine here the sunspot record from 1749 to 1975 using a high-resolution spectral analysis to determine the frequencies of the periodicities, and the amplitude and phase of each line. The analysis is performed with 2724 monthly sunspot values given by Vitinskii[7] and by *Solar Geophysical Data*. The algebraic sign of alternate cycles' sunspot numbers is negative to conform to an assumed 22-yr magnetic reversal cycle.

My analysis is an extension of a method developed by A. Paul[8], which is a very accurate and sensitive method for determining frequencies, amplitudes and phases of unknown lines in a spectrum consisting of both broadband noise and discrete lines. Paul's method differs from conventional FFT methods in that it does not assume that frequencies will be uniformly spaced. The time shift theorem ($\Delta\varphi = 2\pi f \Delta t$) is used to determine the unknown frequencies from the change of phase between the periodogram analysis of segments of the data separated in time.

My analysis uses the additional step of subtracting the waveform corresponding to the approximately known lines in the data, following by iteratively solving for closely spaced lines. The analysis applied to the sunspot record obtains resolution of closely spaced lines having a beat period ($1/\Delta f$) of $1{,}045 \pm 200$ yr. The accuracy of each frequency is more a function of the white noise within the filter bandwidth used in the analysis than of the record length. In this case, the frequency uncertainty is estimated to be $\pm 1/(20T)$ rather than the commonly quoted $\pm 1/(4T)$. The success of the line resolution just mentioned results from the signal-to-noise ratio (line amplitude to Gaussian noise within the bandwidth) of about 25 : 1.

The model is composed of more than 40 lines (Fig. 1) which were determined using more than 100 iterative solutions for one line at a time with all the other lines subtracted from the data. The s.d. of the residuals is 17.0 (unsmoothed monthly sunspots), which is an indication of white noise. Assuming it is uniformly distributed in frequency, the noise within the bandwidth near each line is about 0.6. This has been confirmed by spectral analysis of the residuals. The effect of the noise is difficult to determine; however, the noise certainly degrades the accuracy of the line parameters.

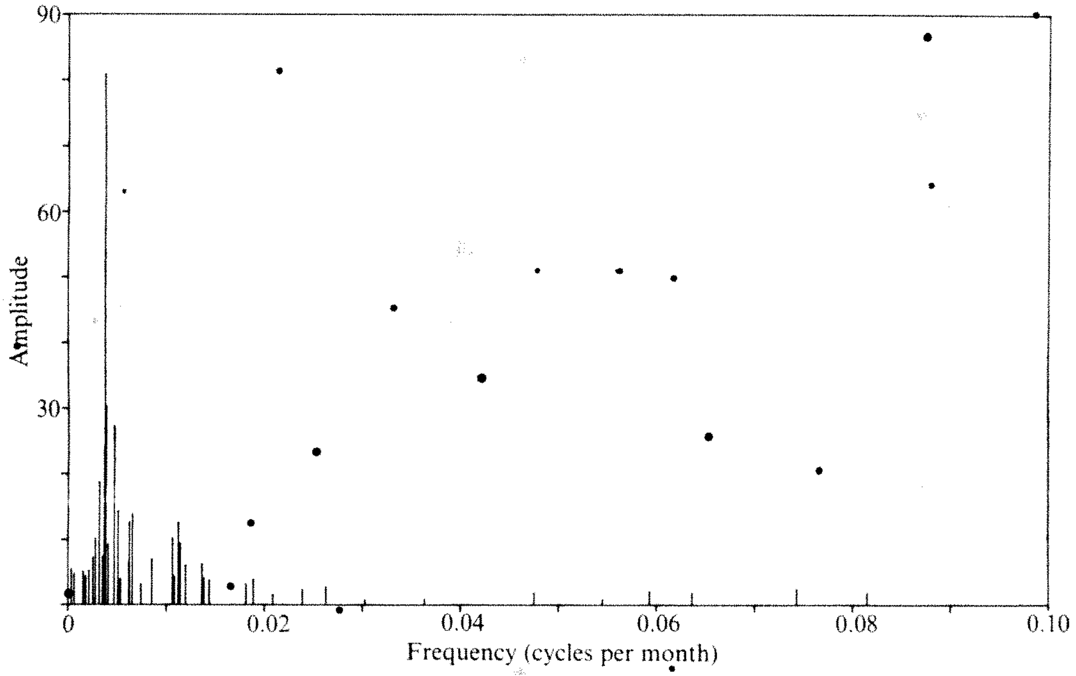

Fig. 1 Spectral lines determined from 227 yr of sunspot data.

The summed Fourier series corresponding to the lines in the model is plotted with the monthly data record in Fig. 2. The series is evaluated beyond the data record at both ends to provide a forecast of future and an aftcast of past solar cycles. Note the striking agreement between the model and observations made between 1740 and 1975.

The fact that the summed series fails to fit the data as accurately in the first and last cycles as in the central cycles may be an artefact of the data window or it may indicate changes in the physical processes of the Sun, for example, changes in the oscillation mode excitations. Figure 3 extends the model sunspot numbers back to 1000 AD and compares with the ^{14}C level given by Suess[9]. The model fails to reproduce the Maunder Minimum, but does show a 1000-yr period envelope, as discussed by Eddy. The closely spaced lines with periods of 21.62, 22.12 and 22.55 yr form a triplet with a beat period of about 1045 yr, having maxima (1112 and 2155 AD) during the same centuries as mentioned by Eddy (about 1175) but a minimum (1724 AD) later than Eddy's (1500–1714 AD). The triplet's minimum is not midway between the adjacent maxima due to the particular phase relationships between the three lines.

Although these results are purely empirical, amounting to data fitting, the stability of the solution and the

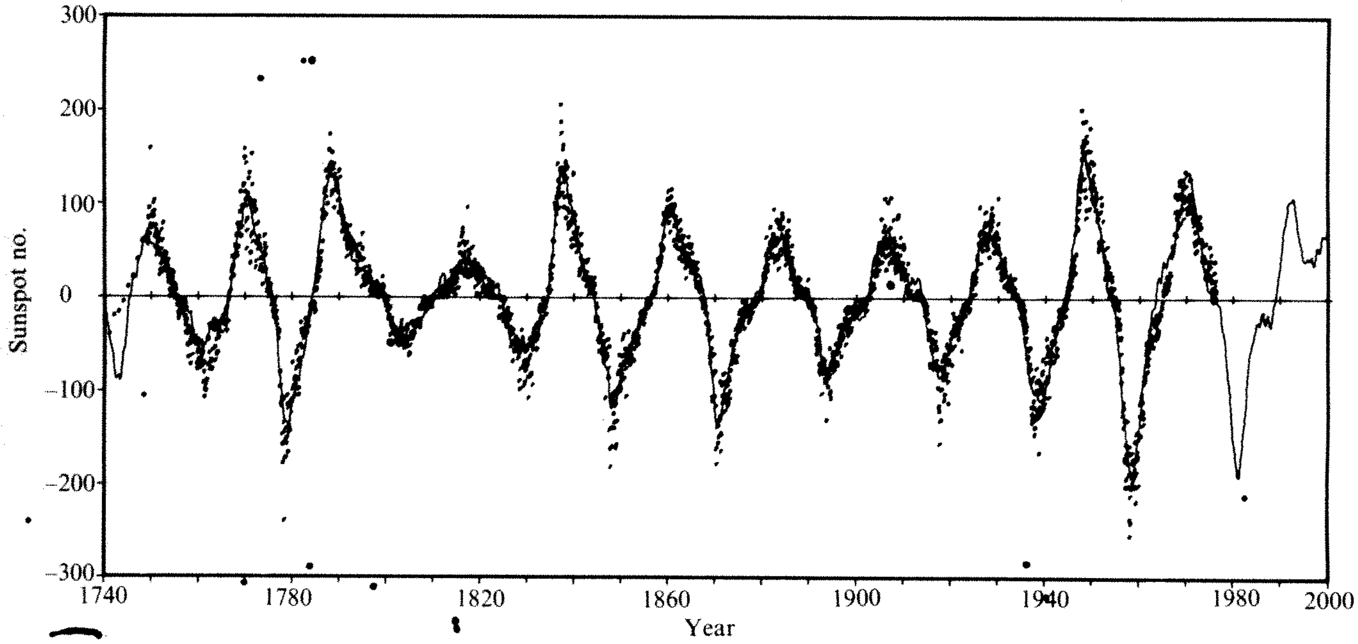

Fig. 2 Observed monthly sunspot numbers (●) and Fourier series model (smooth curve).

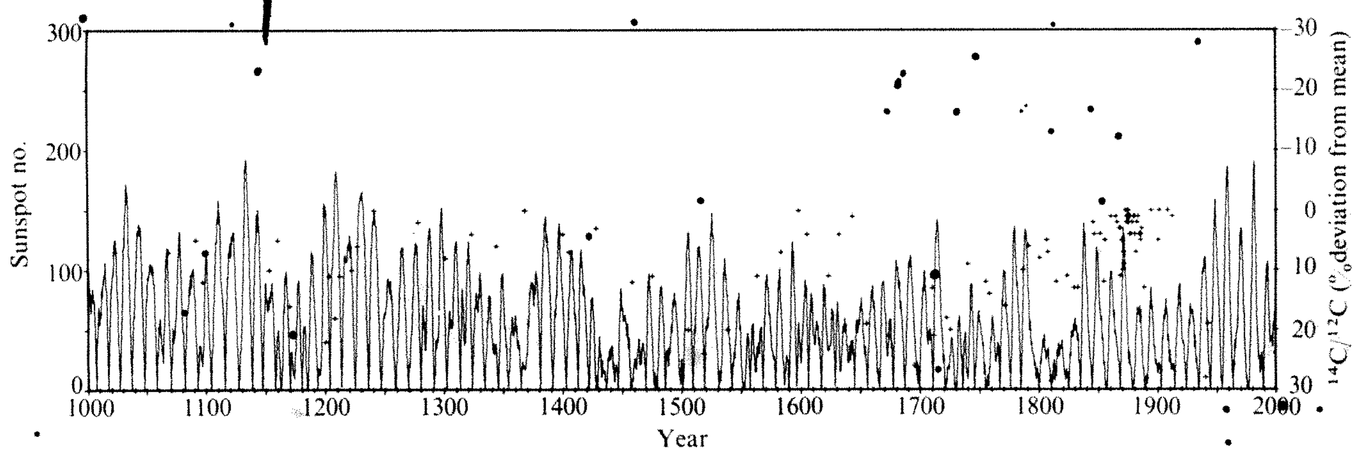

Fig. 3 Fourier Series sunspot model (smooth curve) and ^{14}C : ^{12}C ratio (+, using an inverted scale).

accuracy of the predictions may be an indication of whether Wolff's mode model is correct. My results show that there are lines in the sunspot record as indicated in Wolff's theory. The modes are long-lived, but may persist for centuries instead of millennia. This latter conclusion can be drawn if we attribute the spectral line model's failure to display correctly the Maunder Minimum to changes in physical processes in the Sun.

One area of possible improvement in the analysis is in the choice of data to represent solar activity. Eddy, Wolff and others have used sunspots, which have been counted for a long time. The noise level in the sunspot data (s.d. of 17) is about as expected, however, considering that sunspot groups are arbitrarily assigned a value of ten spots. Another index of solar activity, such as sunspot area or magnetic field intensity, may be a better quantitative measure. Seventy-five yr of high-quality data might yield better results than spectral analysis of 200 yr of lesser quality data.

The fit (Fig. 2) to the sunspot record between 1750 and 1975 is good enough to merit a forecast a few years into the future. Using the zero crossing as an indicator, the model predicts that the next solar cycle began in December 1976. Several authors[4,6,10] have predicted a low sunspot activity level during the next cycle (number 21) with a peak near 50–60. My analysis predicts a larger peak between 130 and 200 during 1981 or 1982. It will be interesting to watch the Sun during the next few years to find out for how long the spectral lines of the model are valid.

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The advancing front of a spreading liquid

A WETTING liquid makes a zero contact angle to a solid, and a drop will spread completely over the surface. There are two stages in the spreading process[1,2]. Initially, the advancing front is pushed from behind by the hydrostatic head of the liquid. As the drop thins out this becomes unimportant and the front is pulled along by the unbalanced surface forces near the perimeter. The shape of the advancing front is not easy to observe without special lighting. In addition, for most liquids it cannot be seen unless precautions are taken to minimise evaporation. I report here microscopic observations which show that the advancing front has a remarkable structure. The moving front does not retain the shape of the original drop. Instead, depending on the liquid, it either assumes a scalloped periodic structure or else moves by a series of random advances, resembling the locomotion of an amoeba, but still with some approximate periodicity.

Figure 1 shows the advancing front for absolute ethyl alcohol spreading on the surface of aluminium that had been evaporated on a glass substrate. (Lighting and precautions for minimising evaporation are described in the legend.) A thin layer of liquid, 1,000–2,000 Å thick, moved ahead of the drop by as much as 1 mm. The drop followed, making a contact angle of about 0.1° to the precursor layer, as determined from the spacing of interference fringes. The front of the thin layer maintained a periodic, almost sinusoidal form as it advanced. This mechanism of spreading seems to be quite general. Similar results were found for a variety of liquids spreading on aluminium–isopropyl alcohol, *n*-heptane, toluene, *n*-octane (all reagent grade) and dimethyl silicone oil (molecular weight 340).

These liquids are all volatile and vapour transport may be an important part of the mechanism by which they spread. Observations were also made of the spreading of a non-volatile liquid, a high molecular weight hydrocarbon-substituted silicone oil, General Electric SF-1147 (mean molecular weight 2,000). It is so non-volatile at room temperature that effects of vapour transport are almost certainly absent. Measurement of the rate of evaporation in vacuum showed that less than one monolayer of molecules evaporates per day at room temperature. In air the evaporation would be even slower. The oil spreads on the surface of evaporated gold. The shape of the advancing front is shown in Fig. 2. Here the spreading was considerably slower than for alcohol due to the difference in viscosities (0.5 P for the oil and 0.014 P for ethyl alcohol). As the liquid spread, convex bulges develop at seemingly random points along the perimeter of the original round drop. These then move ahead and smaller protuberances develop. Though there was considerable randomness, it seems that there is an approximate periodicity here, as before with the alcohol. Again the front advanced far ahead of the bulk of the drop and, after several hours, covered all, or nearly all, of the solid surface.

These observations show that the spreading of a liquid on a solid is a complicated process. Typically, a thin layer moves out away from the rest of the drop, pulled by the unbalanced surface forces near the perimeter. This feature has been noted before[3] from observations of the drop profiles of spreading polymers. The periodic structure indicates an instability. This may be related to the Rayleigh–Taylor instability[4,5] that arises when two immiscible fluids are stratified with the denser fluid above. The Marangoni effect[6] also gives rise to instabilities but it depends on substantial variations of temperature or composition within the fluid, neither of which seems to be present here.

The following simplified picture may contain some of the correct physics. Consider the unbalanced surface force $\Delta\gamma$ at the liquid front

$$\Delta\gamma = \gamma_{\mathrm{SV}} - \gamma_{\mathrm{SL}} - \gamma_{\mathrm{LV}} \qquad (1)$$

The components are the familiar interfacial free energies for the solid–vapour, solid–liquid, and liquid–vapour interfaces. The force acts on the liquid near the interface and pulls it out over the unwetted surface of the solid. Its magnitude is proportional to the length of the liquid–solid–vapour line. In a part of the film of thickness, z, assume that, due to some local inhomogeneity of the solid surface, the front develops a semicircular convex bulge of radius R. The total force pulling outward on the semicircle is $\pi R\Delta\gamma$, whereas before the bulge developed, the force on the same liquid was only $2R\Delta\gamma$. The accelerating force is growing more rapidly than the resisting viscous force and there is a tendency for the front to become as convoluted as possible to take advantage of this. The counter-force is the excess pressure, $\Delta p = \gamma_{\mathrm{LV}}/R$, exerted by a convex curved surface. The pressure, Δp, is exerted over an area πRz and acts to oppose the tendency toward greater convolution. The two opposing tendencies balance when

$$\pi R\Delta\gamma = \pi Rz\gamma_{\mathrm{LV}}/R \qquad (2)$$

This then determines the stable radius of curvature for the undulations in the advancing front

$$R = z\gamma_{\mathrm{LV}}/\Delta\gamma \qquad (3)$$

If this is correct then it may be possible to make a direct measurement of $\Delta\gamma$ since all the other quantities in the equation can be measured.

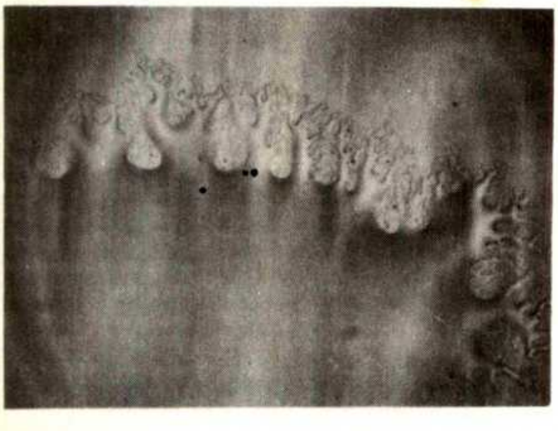

Fig. 1 Advancing front for a drop of ethyl alcohol spreading on the surface of evaporated aluminium. To minimise evaporation the experiment was performed in a cylindrical glass container covered by a glass plate. The bottom of the container was filled with alcohol and the substrate mounted about 1 cm above the surface of the liquid. To apply the spreading drop, the cover was moved aside just far enough to insert a long dropper that reaches nearly to the substrate surface. A drop of about 10^{-3} cm^3 of liquid was dispensed on to the surface and the cover replaced. The drop then spread and the thin advancing front remained stable for many minutes. Vertical illumination with nearly monochromatic light at 5,830 Å was reflected from the metal of the substrate to give interference fringes indicating the thickness of the oil. Successive fringes correspond to a thickness difference of 2,100 Å. The three pictures are for different times after the application of the drop. *a*, 30 s; *b*, 60 s; *c*, 200 s. At *c* the thickness of the advancing front is about 1,000 Å.

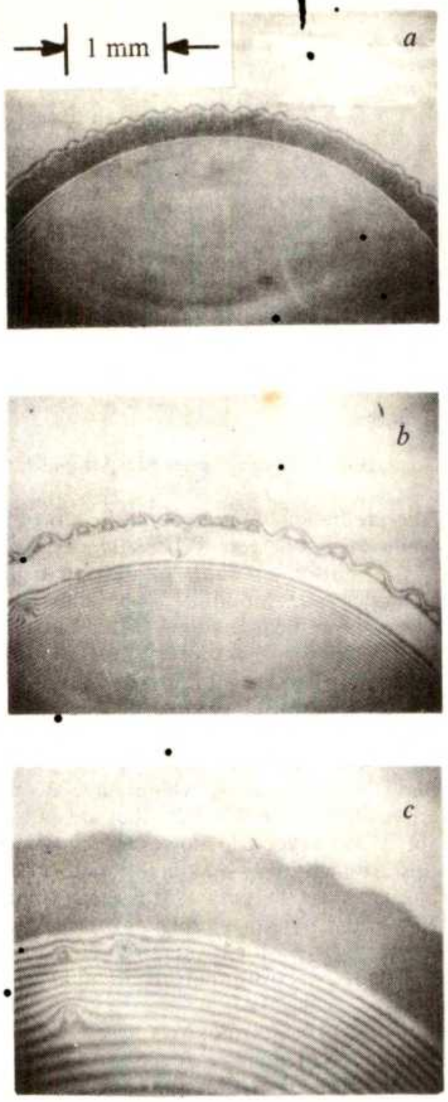

Fig. 2 Advancing front of silicone oil 5 min after application.

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Silane triols of high stability in aqueous solution

ORGANO-SILICON compounds containing silanol groups are highly reactive, undergoing acid- or base-catalysed condensation to form siloxanes even in the presence of surface acid or base, the condensation being more facile in the order

$$\equiv\mathrm{SiOH} < =\mathrm{Si(OH)_2} < -\mathrm{Si(OH)_3}$$

By the use of plastic, quartz or washed borosilicate glass apparatus, several alkyl and aryl silanols and silane diols have been isolated[1–3]. But only aromatic silane triols (R $Si(OH)_3$) have been isolated[3], the corresponding alkyl derivatives being known only as transient species in solution. We report here that certain organo-silane triols display outstanding stability in aqueous solution.

The rate of condensation and polymerisation of silicic acid and organo-silanol derivatives in aqueous solution can be reduced by the addition of polar molecules such as ethers, alcohols and amines and of polymers containing similar groups[4]. The retardation is believed to be due to association through hydrogen bonding between silanol groups and the polar additive. Thus, polyethylene glycols retard the condensation of silicic acid, although inevitably, polymerisation proceeds to the point of gelation or the separation of colloidal silica.

We have prepared silane triols (I–IV), $R.Si(OH)_3$, in which R is an ether-containing moiety, by hydride insertion of the appropriate halo or alkoxy-silane into the allyl ethers of methoxy ethanol, methyl ethylene glycol, methyl trigol and so on, followed by hydrolysis

$CH_3O(CH_2CH_2O)\ CH_2CH_2CH_2Si(OH)_3$ (I)
$CH_3O(CH_2CH_2O)_2\ CH_2CH_2CH_2Si(OH)_3$ (II)
$CH_3O(CH_2CH_2O)_3\ CH_2CH_2CH_2Si(OH)_3$ (III)
$CH_3O(CH_2CH_2O)_{6-7}\ CH_2CH_2CH_2Si(OH)_3$ (IV)

The behaviour of the parent trichloro silyl derivatives of (I–IV) on contact with water revealed notable differences from simple alkyl halosilanes such as CH_3SiCl_3. The latter in water undergoes rapid hydrolysis with evolution of HCl, generation of heat and the rapid precipitation of a gel—essentially methylated silica gel This process is little affected by the presence in the water of, for example, $CH_3O(CH_2CH_2O)_3CH_2CH_2OH$. By contrast, the ether-containing compounds dissolve to give clear solutions for which, in spite of the low pH and raised temperature, low levels of polymerisation are indicated by cryoscopy, cloud point and ^{29}Si nuclear magnetic resonance (NMR).

Fig. 1 Number average molecular weights (cryoscopy) of compound (III).

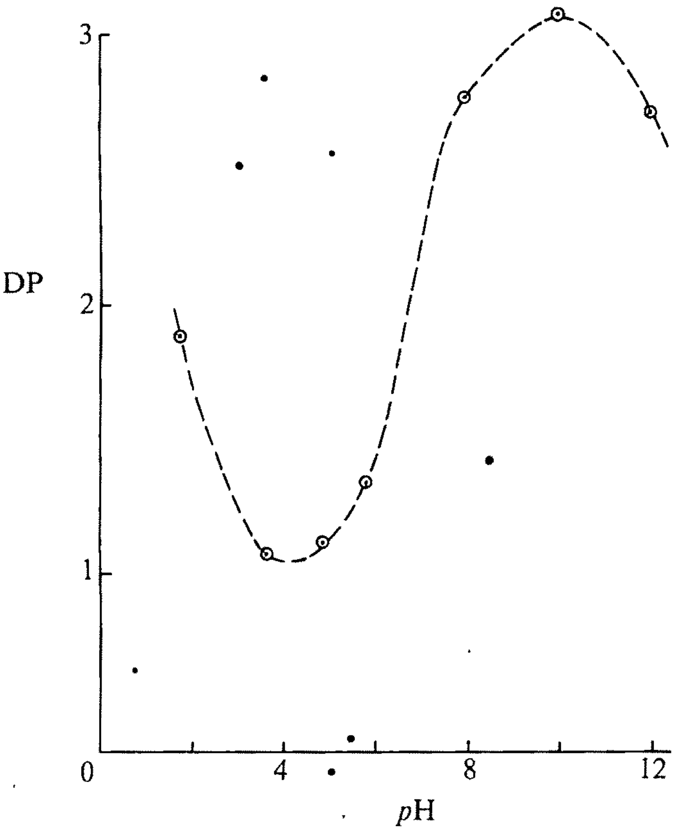

Table 1 Influence of number of ethylene oxide units on stability

Silane triol		Equilibrium DP in 5% (w/w) solution		
		pH 1	pH 5	pH 8
$CH_3O(CH_2CH_2O)CH_2CH_2CH_2Si(OH)_3$	(I)	7.2	2.4	7.1
$CH_3O(CH_2CH_2O)_2CH_2CH_2CH_2Si(OH)_3$	(II)	—	1.9	—
$CH_3O(CH_2CH_2O)_3CH_2CH_2CH_2Si(OH)_3$	(III)	1.9	1.1	3.4
$CH_3O(CH_2CH_2O)_{6-7}CH_2CH_2CH_2Si(OH)_3$	(IV)	2.9	1.1	1.6

DP, degree of polymerisation.

Figure 1 shows the number average molecular weights (cryoscopy) of (III) in 5% (w/w) aqueous solutions as a function of pH within the range 2–12 (after several days equilibration) when the highest degree of polymerisation found was only about 3.

The equilibrium molecular weights for (I–IV) (5% in water) at various pH values (Table 1) clearly illustrate that (I) and (II) are more susceptible to polymerisation than (III) and (IV), which contain the more ether groups.

The cloud point of a 5% solution of (III) did not change from the original value following 4.5 h of heating at 92 °C, whereas that of a 5% solution of (I) or (II) showed a reduction, indicating an increase of molecular weight.

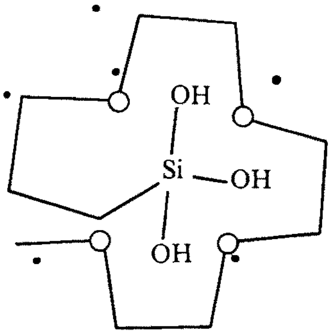

Fig. 2 Possible configuration to give stability.

The essentially monomeric nature of (III) in water was confirmed by ^{29}Si NMR. At pH 5–6 a 5% (w/w) solution contained only a trace of dimer while a 50% (w/w) solution still contained 75% monomer and only 25% dimer with no oligomers. An aqueous solution of $CH_3O(CH_2CH_2O)_3CH_2CH_2CH_2Si(OH)_3$ (III) evaporated to dryness gave a clear, viscous, water-soluble oil with a degree of polymerisation (cryoscopy) of about 7. ^{29}Si NMR showed this oil to contain no monomer or dimer and to undergo slow depolymerisation in water over several weeks.

The results suggest that by incorporating an ethylene oxide function by means of an Si–C bond into a silane triol, high solution stability can be attained. At pH 5, for example, at which the polymerisation of a silane triol is minimal and hydrogen bond formation near-optimal, the equilibrium degree of polymerisation reaches unity when four ether-oxygen atoms are involved. This stability could arise from the fact that the oligomers are soluble and hence, equilibrium would be in favour of depolymerisation. Compounds (I–IV), however, are all adequately soluble for this to apply and yet degree of polymerisation becomes unity only with (III).

This suggests that stability arises from full shielding of the silanol groups by intramolecular hydrogen bonding with the ether-oxygen atoms A possible configuration (ignoring interposed water molecules) is shown schematically in Fig. 2. A stable, associated pair can also be envisaged in which the silane triol head group of each molecule is embedded in the ethylene oxide chain of the other.

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Palaeomagnetic field intensity variation recorded in a Brunhes epoch deep-sea sediment core

DEEP-SEA sediments have been shown to possess a natural remanent magnetisation (NRM) that often can be attributed to the statistical alignment of detrital magnetic grains in the Earth's magnetic field at or shortly after the time of their deposition[1,2]. In favourable circumstances this remanence can be interpreted as a record of palaeomagnetic field behaviour[3,4] In the study reported here we have attempted to describe relative variations in palaeomagnetic field intensity on a time scale of 10^4–10^5 yr, during the past 700,000 yr, using the palaeomagnetic record of deep-sea sediment piston core RC10-167 (33°2′N, 150°23′E), which has an exceptionally thick section of sediment deposited during the Brunhes normal polarity epoch[5] (Fig. 1). After subtracting the stratigraphic contribution of several distinct volcanic ash layers interspersed with the otherwise uniform pelagic sediment[6], we calculate an average deposition rate of 2.1 cm kyr^{-1} between the adjusted level (1,470 cm) of the Brunhes–Matuyama boundary (t=700,000 yr) and the top of the core, assumed t=0 yr. A 2-cm thick sample, representing about 1,000 yr of deposition, was taken at an average interval of 3.3 cm (representing about 1,600 yr). This sampling placed a theoretical limit of 3,000–4,000 yr on the period of a resolvable sinusoidal variation.

Progressive alternating field (AF) demagnetisation of the NRM in 12 pilot samples showed consistent directions of magnetisation, to within 10° of the initial value, with demagnetisation up to 400 Oe. The mean remanent inclination after 150-Oe AF is 51.2°, close to the expected dipole inclination (52.4°) for the core site latitude. The median demagnetising field (MDF; peak AF required to remove half the initial remanence) is high and relatively uniform, averaging 270±40 Oe (s.d.). The total magnetic coercivity distribution was investigated by progressive AF demagnetisation of an anhysteretic remanent magnetisation (ARM; imparted in a 1.0-Oe direct field coaxial with a 1,000-Oe AF) and an isothermal remanent magnetisation (IRM; imparted in a 1,000-Oe direct field). The average MDF values for the 12 specimens are 230±15 Oe for ARM and 170±25 Oe for IRM.

On the basis of these experiments we conclude that: (1) the NRM has a high magnetic stability with little contribution from unstable magnetic components; (2) the small scatter in MDF values for NRM, ARM and IRM shows that the magnetic coercivity spectrum is quite uniform over the length of the core; (3) the high stabilities of remanence and the relative stabilities of ARM and IRM[7] are indicative of fine-grained magnetic carriers; (4) the coercivity spectrum of NRM is more closely approximated by ARM than by IRM in this sediment.

We assume that the magnetisation acquisition process was the same at each stratigraphic level in the sediment and that the intensity of NRM is only a function of palaeofield strength and the magnetic mineral concentration. The variation with stratigraphic level in the sediment (hence age) of NRM intensity, normalised to take into account fluctuations in magnetic mineral concentration, then might provide a record of relative variations in palaeomagnetic field intensity. We do not deal explicitly with the still poorly understood details of the magnetisation acquisition process in the sediment, and so absolute values of palaeofield strength are not obtained directly by this method. A similar approach has been used to describe palaeofield intensity variations across magnetic polarity transitions in deep-sea sediment cores[8,9].

Because of our conclusion (4) above, we have chosen to use the intensity of ARM as a relative measure of the quantity of magnetic mineral carrying the NRM. The variation with depth in this core of ARM, however, is similar to that of initial susceptibility (Fig 1). Together they provide an internally consistent record of fluctuating magnetic mineral concentration. This correspondence supports our inference of a coercivity spectrum that is uniform over the core length since the magnitude of ARM and initial susceptibility each are affected preferentially by different magnetic grain size ranges. By assuming that over this sediment core the intensity of NRM is uniformly and directly proportional to the strength of the magnetising field[1,10] and to magnetic mineral content as measured by ARM[11], the ratio of NRM/ARM should be directly proportional to palaeofield strength alone. In practice, we have used the ratio after partial demagnetisation of each remanence in 150-Oe AF, to remove any possible unstable magnetic components.

A plot of the ratio of NRM/ARM (partially demagnetised) in the Brunhes section of core RC10-167 is shown in Fig. 2. The data have been interpolated linearly to 512 equally-spaced values (from the 448 samples measured), giving an interpolated interval of 2.9 cm (or about 1,400 yr). This was done to facilitate further analysis of the data. The ratio of NRM/ARM has a variation of as much as 100%, but more typically about 50%, about a mean value of 0.10 in the core. The record is nearly stationary as the linear trend is only −0.0036 for 1,470 cm (or 700,000 yr). Visually, three wavelength components seem to dominate the record. a broad baseline fluctuation with an apparent wavelength of about half the record length, a variation with an apparent wavelength of about 1 m which gives the record its dominant character, and last, a short wavelength component that seems to ride on the longer wavelength variations.

A power spectrum analysis was performed on this record to identify better any periodic components. Power spectra were estimated using a direct (fast Fourier transform) method with a cosine-rectangular window, and finally smoothed with a three-point Hanning filter[12] (Fig. 2). We observe an initial falloff in power to a wavelength of approximately 25 cm beyond which the spectrum is flatter and approximates the spectrum of white noise. This change in overall slope in the power spectrum may be a function of the remanence acquisition mechanism in these sediments, the periodic content of geomagnetic intensity variations or a combination of these. For example, deep-sea sediments may not acquire a stable remanence at the instant of deposition but rather at, or more importantly, over some depth below the sediment–water interface[1,2]. The record of palaeomagnetic field variations with periodicity of the order of the time in which stable remanent magnetisation is acquired will tend to be obscured. On the other hand,

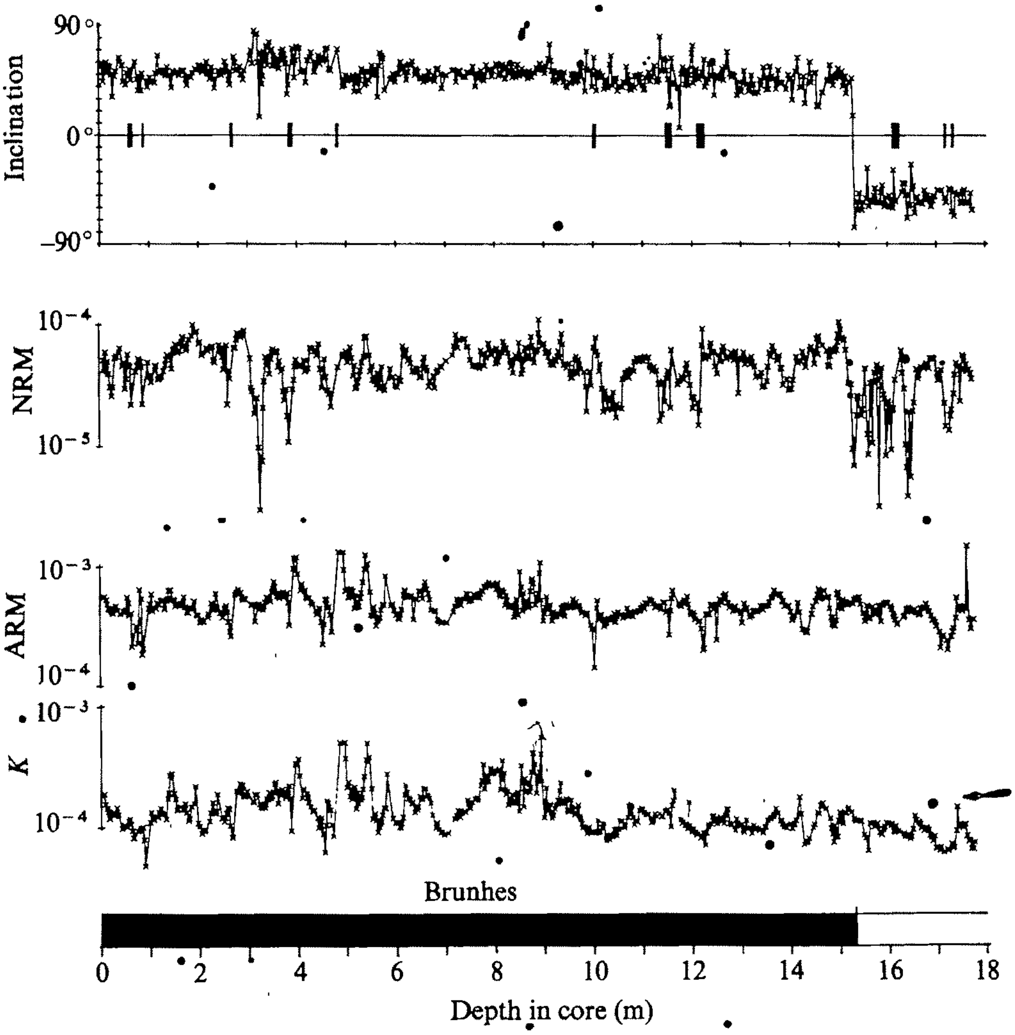

Fig. 1 Inclination direction and intensity of natural remanent magnetism (NRM) against depth in deep-sea sediment core RC10-167. Change in sign of inclination at 15.3-m depth is interpreted as the Brunhes–Matuyama magnetic reversal boundary[5]. Solid bars on axis of inclination plot are the positions of volcanic ash layers[6]. Also shown is the intensity of ARM and the bulk susceptibility (k). The direction and intensity of NRM and the intensity of ARM are after AF demagnetisation in 150 Oe. Magnetisation units are in c.g.s. g^{-1}.

sediment cores with higher deposition rates will need to be studied to determine the contribution of geomagnetic field intensity variations to the shorter wavelength (less than about 12,000 yr) portion of the spectrum

The power spectrum is dominated by periodic components with wavelengths of several metres and greater, and a component that forms a more coherent peak centred at a wavelength of about 90 cm. We hesitate to ascribe the longer wavelength variation in NRM/ARM to that of palaeomagnetic field intensity. Our record is too short to evaluate such an apparent long period variation and it may reflect some long term lithological or diagenetic control on the remanent magnetism of the sediment that was not effectively normalised by the procedures used. On the other hand, because of its better defined and persistant character in the record, we feel more confident in interpreting the 90-cm wavelength variation in NRM/ARM as a previously undescribed component of secular variation of palaeointensity. Its apparent period is about 43,000 yr.

A bandpass filter centred on this wavelength (Fig. 3) shows that this component has about a 30% variation about its mean value, an amplitude of magnitude similar to that obtained for palaeointensity variation[13]. We note, however, that our interpreted palaeointensity variation has a period about four times greater than previous estimates[14]. This apparent discrepancy may be due to an inadequate definition of variations in the relatively short archaeomagnetic record on which previous estimates of palaeointensity periodicity have been made. On the other hand, it may simply reflect a more complicated variation in palaeointensity than previously suspected. The cause of this discrepancy is difficult to resolve, particularly because our record seems to lack sufficient resolution to detect the shorter periodicity of palaeointensity variation (about 10,000 yr) estimated from the archaeomagnetic record.

The long period and relatively large amplitude of the inferred 43,000-yr palaeointensity variation suggest that it is due primarily to a secular variation in the strength of the main dipole field, and consequently must reflect processes within the Earth's core associated with its generation. Malkus[15] has proposed that the geomagnetic dynamo may be driven by forces arising from the precession of the Earth's rotational axis. The efficacy of this energy source in driving the geomagnetic dynamo has been questioned by some[16,17] while others[15,18] maintain that it is the basic driving mechanism (although convection may play some part). The Earth's rotational axis precesses about the normal to ecliptic with a period of about 21,000 yr. The angle between the rotational axis and the normal to the ecliptic (the obliquity), however, does not stay constant but varies between approximately 22.1° and 24.5°, with an average period city of about 41,000 yr (ref. 19). The precessional force on the core can be expected to vary with the obliquity. If precession has a role in driving the geomagnetic dynamo, some sympathetic variation with obliquity might be expected in the Earth's magnetic field. We suggest that the apparent 54,000-yr variation in palaeointensity recorded in RC10-167 is near to the periodicity of obliquity, and reflects the modulation of a precessionally-driven dynamo by changes in the obliquity.

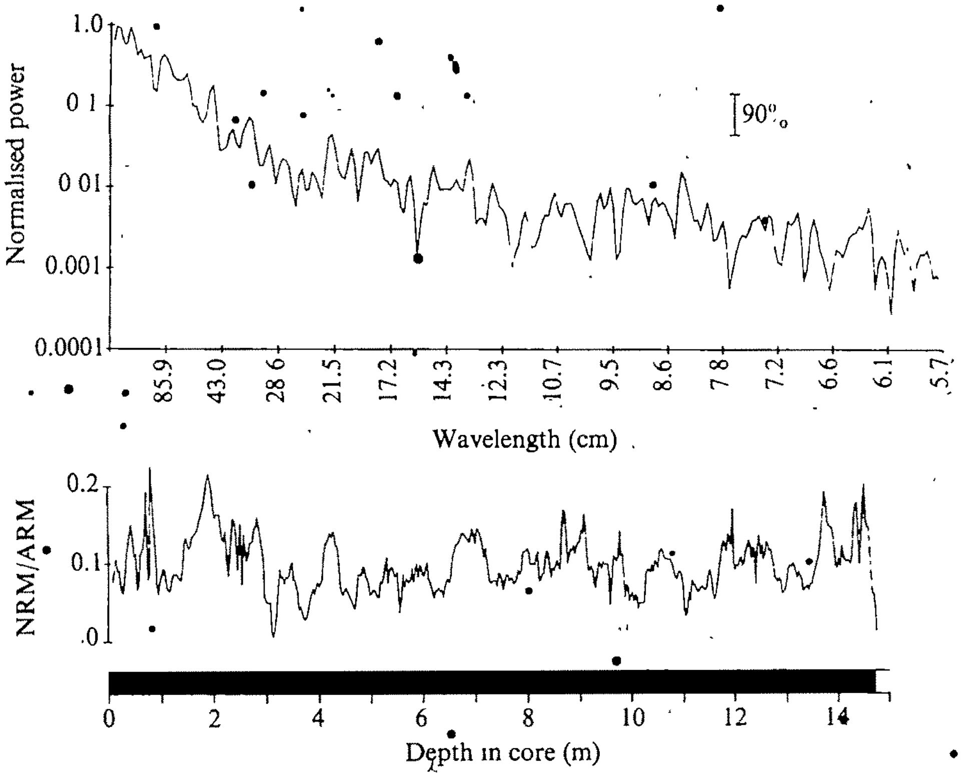

Fig. 2 Lower trace shows the ratio NRM/ARM, against ash-free depth in core RC10-167. The initial set of 448 sample measurements has been interpolated linearly to produce 512 equally-spaced values. Upper trace shows power spectrum of NRM/ARM variation record shown in lower trace, using the fast Fourier transform with a cosine-rectangular window. Estimates of power spectra were smoothed further by a three-point weighted (Hanning) filter. The solid bar is an estimate for the 90% confidence interval

Although the magnitude of precessional force may vary by only less than 10% between times of maximum and minimum obliquity[20], much less than the variation in palaeointensity observed here, the relationship between the magnitude of precessional force and the field generated may not be simple. It is possible that some critical amount of energy is required to sustain dynamo action, as found in laboratory dynamo models[21]. Near this critical value, relatively small variations in contributing forces, such as precession, may have pronounced effects in the magnitude of the generated magnetic field. Figure 3 shows the variation with time in the Earth's obliquity[19] and in inferred geomagnetic palaeointensity as recorded in core RC10-167, assuming a linear time scale between the top of the core and the level of the Brunhes–Matuyama boundary. There seems to be a good correspondence in the expected sense between the palaeointensity and obliquity variations over this time, with obliquity maxima apparently preceeding the inferred intensity maxima by about 5,000 yr.

There is no statistical compulsion to accept at the 90%

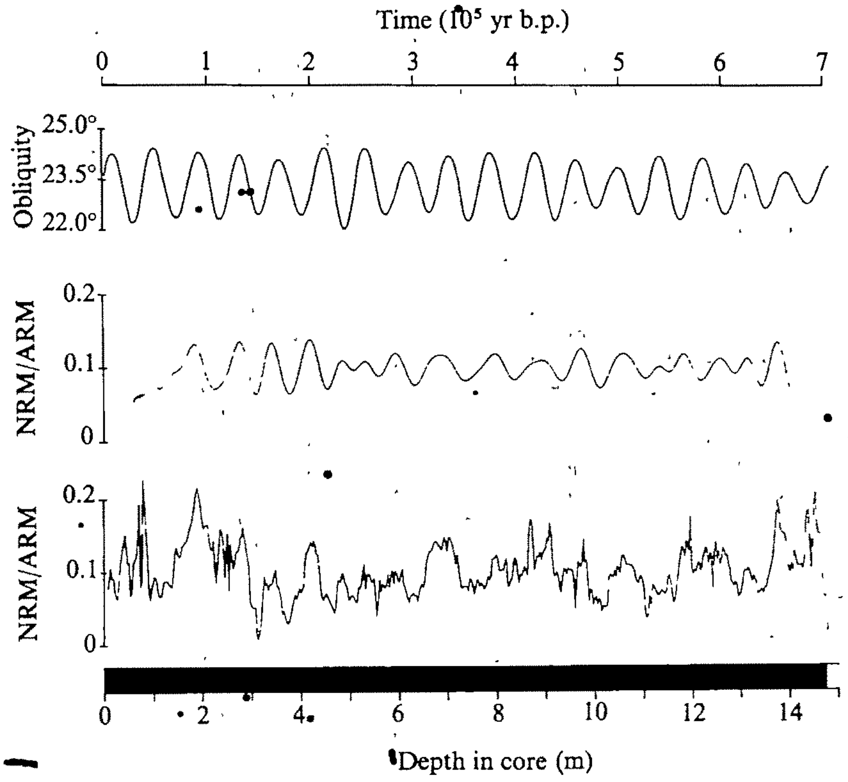

Fig. 3 The variation of NRM/ARM in core RC10-167 (bottom) and the same record (middle) filtered with a cosine-tapered band-pass centred at a wavelength of 90 cm The time scale is based on the assumption of a uniform deposition rate (volcanic ash layers excluded) between the top of the core ($t = 0$ yr) and the level of the Brunhes–Matuyama magnetic reversal boundary ($t = 700{,}000$ yr). Also shown (top) is the variation with time in the Earth's obliquity from ref. 19.

confidence level the existence of the 43,000-yr period in the NRM/ARM record on the basis of its power spectral density alone. We have some confidence in its reality, however, on the strength of two arguments—the apparent phase coherence with the variation in the Earth's obliquity and the knowledge that such a correlation is not physically absurd We recognise, however, that results from a single sediment core should be viewed with caution, especially when a global phenomenon is invoked in explanation. It is possible that the inferred palaeointensity variation in this core reflects a more local palaeomagnetic field behaviour or a physical property of the sediment, in spite of the magnitude and internal consistency of the observed NRM/ARM variation. Other periodicities of palaeofield intensity may also be involved These may be difficult to identify with certainty in this core because of the limited record length available to characterise any longer period variations, and because of the restriction imposed by the sampling interval and sediment deposition rate in resolving with confidence any shorter period variations. Nevertheless, we feel that the data described here provide sufficient basis for serious consideration of a 43,000-yr variation in the palaeomagnetic field intensity during the past 700,000 yr. Confirmation of the existence of such a variation is being sought in a deep-sea sediment core of different lithology and geographically remote from RC10-167. We point out, however, that of approximately 1,000 cores that we have studied palaeomagnetically from the L-DGO core collection, RC10-167, is unique in having the longest complete record of the Brunhes normal magnetic epoch

If the apparent correlation between variation in geomagnetic palaeointensity and the Earth's obliquity is substantiated by further work, several implications are apparent. First, it would provide evidence that forces arising from the Earth's precession contribute to driving the geomagnetic dynamo Second, there is now good evidence that long term climatic variations have been controlled, at least in part, by the Earth's orbital parameters[22] Therefore, a correspondence between some component of climatic change and variations in the Earth's magnetic field can be expected, due to a possible common mechanism

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Growth of *Pinus pumila* and climate fluctuation in Japan

Tree-ring analysis shows a relationship between annual ring growth and climatic changes in the United States[1–3] and in the Scandinavian countries[4] We have measured annual growth in terms of elongation of the terminal shoot of dwarf pine, *Pinus pumila* (Pallas) Regel, which is common in the alpine zone of Japan and eastern Siberia. This tree was used as the indications of annual growth are clearer than in other plants The ages of branches used for measurement are 50–200 yr, although dating at >100 yr is difficult We discuss here the relationship between the fluctuation in annual elongation of dwarf pine and climatic changes in Japan or in higher latitude of Northern Hemisphere

Elongation of the terminal bud of *Pinus pumila* starts in early June and terminates in late July The buds do not elongate further in the following years. Thus annual elongations of the past years are measurable and the number of years so indicated agrees with the number of annual rings at the node. Annual elongation of the main shoot of the pine was measured on trees in many locations of the alpine zone of Japan There was uniformity of annual variations in elongation among most of populations of different locations (Figs 1 and 2) Such a uniformity was even observed between the populations of Mt Hakusan and Mt

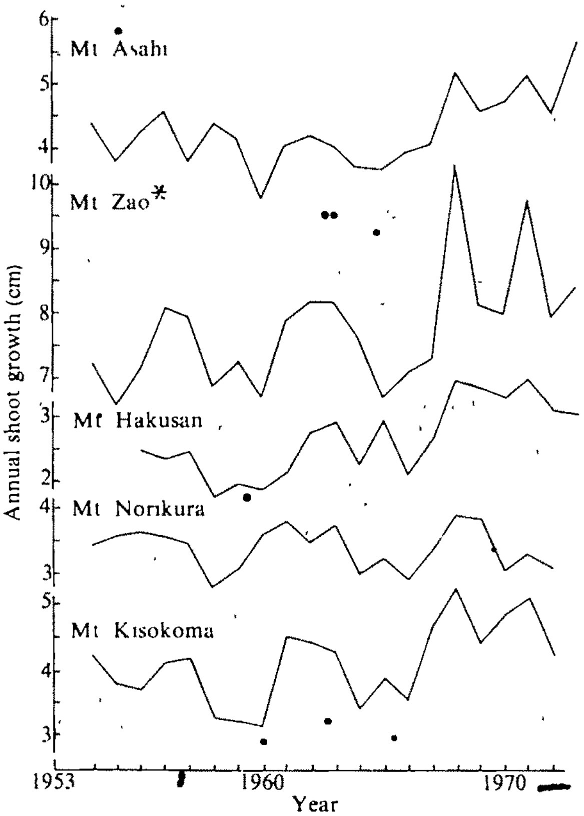

Fig. 1 Annual shoot growth of *Pinus pumila* in five stands of the alpine zone of Japan. Values for shoot growth are means of 60–20 plants The stands are shown in Fig. 2. Correlation coefficient between each two stands. Mt Kisokoma–Mt Norikura, $r = 0.396$; Mt Kisokoma–Mt Hakusan, $r = 0.873$; Mt Kisokoma–Mt Zao, $r = 0.785$; Mt Kisokoma–Mt Asahi, $r = 0.701$; Mt Norikura–Mt Hakusan, $r = 0.362$; Mt Norikura–Mt Zao, $r = 0.325$; Mt Norikura–Mt Asahi, $r = 0.072$; Mt Hakusan–Mt Zao, $r = 0.733$; Mt Hakusan–Mt Asahi, $r = 0.674$; Mt Zao–Mt Asahi, $r = 0.768$. *Hybrid population of *P. pumila* and *P. parviflora*.

Asahi, though these two mountains are about 950 km apart. This result suggests that the growth of the pine is affected by climatic factors that predominate over a wide region of Japan.

Annual elongation of the pine of Mt Kisokoma correlated positively with the total sunshine in June, July and August of the previous year, and/or with the mean temperature of June, July and August of the previous year (Fig. 3). There is presumably some dependence of growth on rain or snowfall Correlation between growth and sunshine, however, is apparent even if effects of rainfall are ignored.

There are many reports on climatic changes of the Earth[5–7]. We measured the annual elongation of the old pine (about 200 yr old) at Mt Norikura going back to 1890, and its fluctuation was compared with the data on fluctuation of temperature around the world shown by G. S. Callendar (Fig. 4). In Fig. 4, the temperature of sub-arctic zones around 1890–1900 is shown as low and that around 1940–1950 as high; elongation of the shoot was small around 1900–1910, and large around 1940–1950. Thus fluctuation in elongation varies with temperature in the sub-arctic (60–73 °N) with a slight time lag. This coincidence with temperature fluctuation in the sub-arctic zone rather than in Japan is of note. A similar relationship was seen in other population of *Pinus pumila* with other data of temperature fluctuation in the Northern Hemisphere (data of J. M. Mitchell). Thus the growth of the pine in the alpine zone of Japan accurately reflects the fluctuation of temperature in the Northern Hemisphere, especially in the sub-arctic zone.

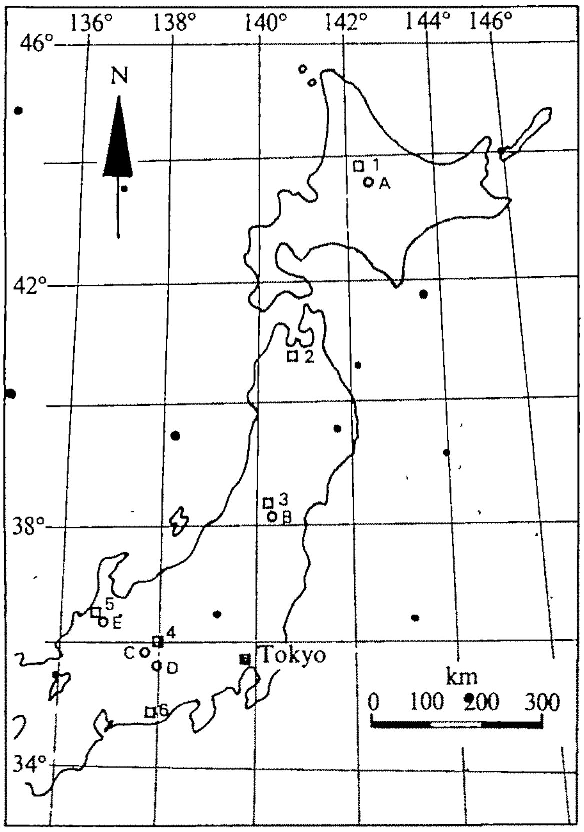

Fig. 2 The location of the sample stands of *P. pumila* and the meteorological stations where climatic data was collected. A, Mt Asahi; B, Mt Zao; C, Mt Norikura; D, Mt Kisokoma; E, Mt Hakusan; 1, Asahikawa; 2, Aomori; 3, Yamagata; 4, Matsumoto; 5, Kanazawa and 6, Hamamatsu.

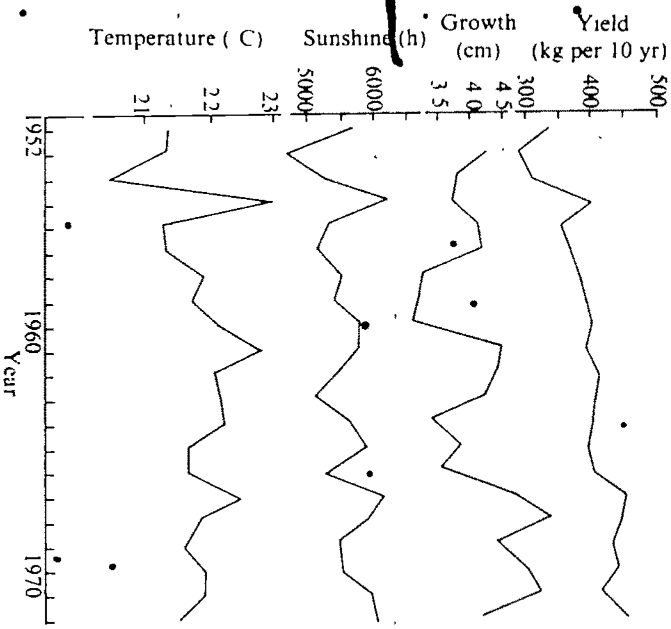

Fig. 3 Fluctuation of annual shoot growth of *Pinus pumila* of Mt Kisokoma in Japan and fluctuation of temperature (mean of June, July and August at six stations) and sunshine (sum of June, July and August at six stations) and mean rice yield per acre in Japan[8,9]. Each value of shoot growth is the mean of 40 plants. The six meteorological stations are shown in Fig. 2. Correlation and regression coefficients: (1) Temperature of previous year (x)–shoot growth (y), $r = 0.291$, $Y(\text{cm}) = 0.303x(°\text{C}) + 2.538$. (2) Sunshine of previous year (x)–shoot growth (y), $r = 0.407$, $Y(\text{cm}) = 0.001x(\text{h}) + 0.726$. (3) Rice yield previous year (x)–shoot growth (y), $r = 0.518$, $Y(\text{cm}) = 0.007x(\text{kg per 10 a}) + 1.306$. (4) Sunshine ($x$)–temperature ($y$), $r = 0.636$, $Y(°\text{C}) = 0.001x(\text{h}) + 17.080$. (5) Sunshine ($x$)–rice yield ($y$), $r = 0.685$, $Y(\text{kg per 10 a}) = 0.072x(\text{h}) - 7.714$. (6) Temperature ($x$)–rice yield ($y$), $r = 0.552$, $Y(\text{kg per 10 a}) = 43.207x(°\text{C}) - 549.355$.

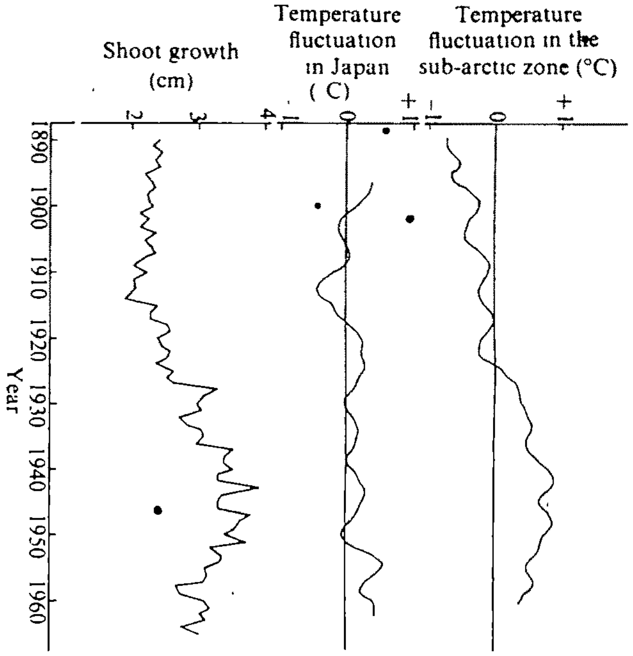

Fig. 4 Fluctuation of annual shoot growth of *Pinus pumila* of Mt Norikura in Japan and fluctuation of temperature in Japan and the sub-arctic (60–73°N) zone Values of shoot growth are the means of 33 plants. Climatic data from G. S Callendar[6]. Correlation coefficient and regression equations: (1) Temperature in the sub-arctic zone (x)–pine growth in Mt Norikura (y), $r = 0.835$, $Y(\text{cm}) = 0.895x(°\text{C}) + 2.616$. (2) Temperature in Japan (x)–pine growth in Mt Norikura (y), $r = 0.396$, $Y(\text{cm}) = 0.982x(°\text{C}) + 2.687$. (3) Temperature in the sub-arctic zone (x)–temperature in Japan (y), $r = 0.298$, $Y(°\text{C}) = 0.144x(°\text{C}) + 0.048$.

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Annual fluctuation of the pine elongation in Mt Kisokoma was also compared with annual fluctuation of the mean yield per acre of rice in Japan (Fig. 3). Rice yield per acre in Japan is affected mainly by sunshine and temperature of June, July and August. A similar trend of fluctuation is seen between pine growth and rice yield per acre of the previous year.

Study of *Pinus pumila*, may thus be useful in the estimation of past environmental changes such as fluctuations of temperature and sunshine.

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Strong attenuation of Rayleigh waves in Tibet

THE Tibetan Plateau, which has an average elevation of 5.0 km over 7×10^5 km^2, is the largest topographic mass above sea-level on the Earth. Because it is covered with Cretaceous limestones[1] its uplift must be Tertiary, and is probably related to the Himalayan continental collision. There is considerable debate concerning its present structure and mode of formation. Various authors have theorised that Tibet was uplifted by underthrusting of a second crustal layer[2–6]; by horizontal compression and thickening[7–9], or by low-density material in the mantle[10]. All that is known with certainty is that it is isostatically compensated[11], and covered with widespread Neogene calc-alkaline volcanic rocks[12]. The study of Rayleigh wave attenuation reported here indicates that the lowermost part of the crust is partially molten, and that uplift has been due to horizontal compression.

Because of its size, uniformity and difficult access, Tibet is most easily studied using seismic surface waves. Rayleigh waves have the advantage that they are not confused with Love waves even if horizontal refraction causes the waves to arrive from an unknown direction. Because there are no long-period stations north of Tibet, however, the attenuation cannot be measured by using two stations on one raypath. Instead, we have compared Tibetan paths with other reference paths that do not cross the plateau (Fig. 1). By normalising the wave spectra at short periods, the azimuthal factor in the source spectrum can be cancelled approximately, and the spectral differences at longer periods can be ascribed to attenuation or scattering. We chose a period of 20 s for normalisation, because it is the average spectral peak on the seven reference seismograms. This method gives only a minimum value for attenuation, but records from the nuclear explosion of October 14, 1970 at Lop Nor give similar results without any normalisation.

Data from six earthquakes (yielding 14 Tibetan paths) are shown in Fig. 2. They were obtained by digitising the long-period vertical records from WWSSN stations at Kabul, Nilore, Lahore, New Delhi, Shillong and Hong Kong. Before differencing, the raw spectra $A(\omega)$ were smoothed by the operation:

$$\overline{A}(\omega) = \frac{\int_0^\infty A(\xi) \exp\left(-50\left(\frac{\xi-\omega}{\omega}\right)^2\right) \mathrm{d}\xi}{\int_0^\infty \exp\left(-50\left(\frac{\xi-\omega}{\omega}\right)^2\right) \mathrm{d}\xi}$$

On 11 of the 14 paths across Tibet, a large reduction in the amplitude of long period (40–50 s) Rayleigh waves is evident. This cannot be due to differences in the instruments (they are identical) or in the source spectrum or path length. They must result from different velocity structures under the stations, scattering or attenuation in Tibet.

The effect of the first is probably minimal, because all stations except Kabul are on stable continental crust of about 40-km thickness[13,14]. Selective reflection of long periods at the boundaries of Tibet is probably more important. The plateau is, however, bounded by mountain ranges (Himalayas and Kun Lun) in which the crustal thickness change is spread out over more than one wavelength. For such dipping interface problems, it has been found that reflection of Love surface waves is very small[15], so we do not believe that this effect accounts for the large spectral differences. These have been interpreted as the result of attenuation, so that:

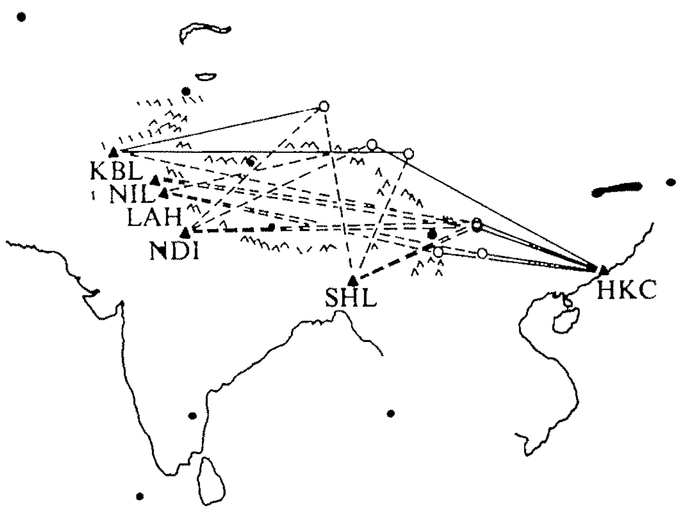


Fig. 1 Surface wave paths used to study attenuation. The Tibetan Plateau is outlined with mountain symbols; ○, earthquakes; ▲, stations, – – –, $Q_R(40) < Q_R(20)$; . . . . . ., $Q_R(40) > Q_R(20)$; ———, reference paths.

$$\min(Q_R^{-1}) = \frac{PG}{\pi\Delta} \ln\left[\frac{\overline{A}(\omega)\,\overline{A}_R(0.05)}{\overline{A}_R(\omega)\,\overline{A}(0.05)}\right]$$

where Q_R is the Rayleigh elastic quality factor, P is period, G group velocity, Δ the path length, and $A_R(\omega)$ the spectrum on the reference path.

The minimum attenuation obtained (Fig. 3) has a peak at 50-s period. The half-width of the peak is a factor of 2.8 in frequency, which is too narrow to be produced by frequency dependence of a geologically complex medium with various relaxation times, but is more likely to result from an increase of attenuation with depth, so that longer-period waves are more

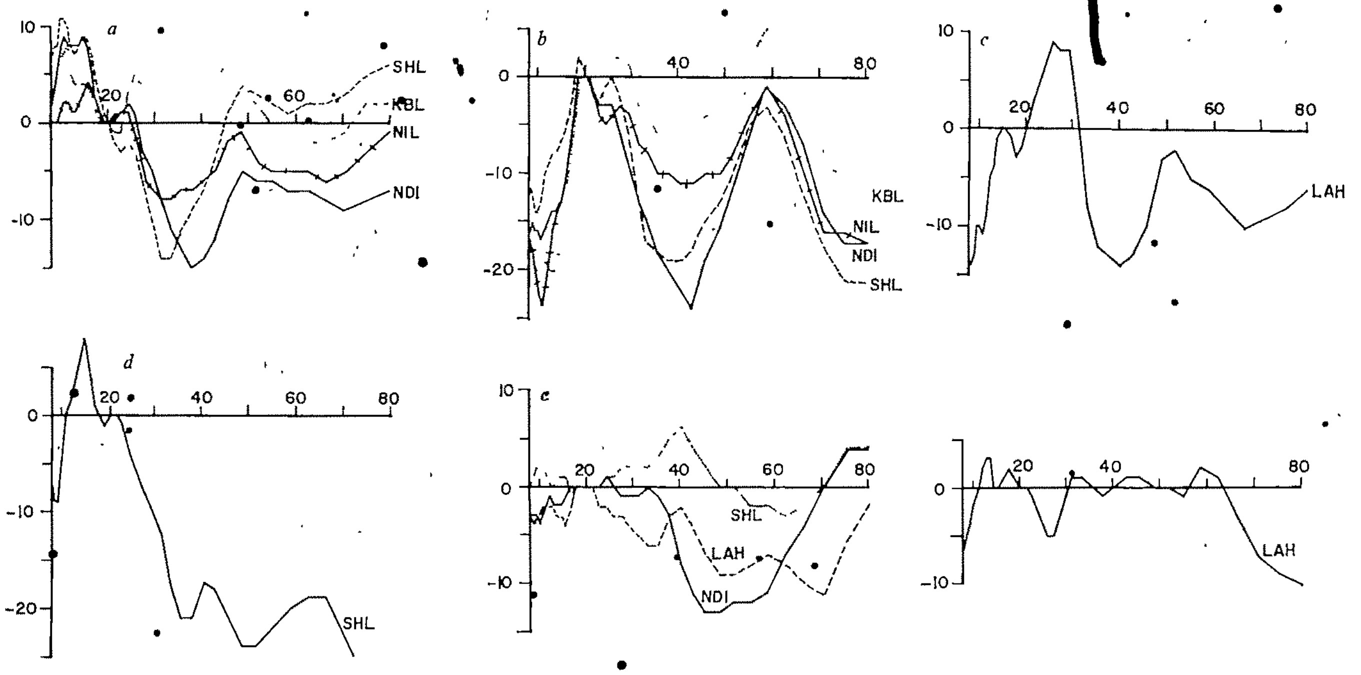


Fig. 2 Power spectrum differences in dB between Rayleigh waves crossing Tibet and those on reference paths from the same source. Period in s is given on the horizontal scale. The difference is set to zero at 20 s to normalise each curve. *a*, August 16, 1971 (1854 h), ref. HKC. *b*, August 16, 1971 (2237 h), ref. HKC *c*, February 5, 1966, ref. HKC. *d*, March 24, 1971, ref. KBL. *e*, March 16, 1964, ref. HKC. *f*, September 28, 1966, ref. HKC.

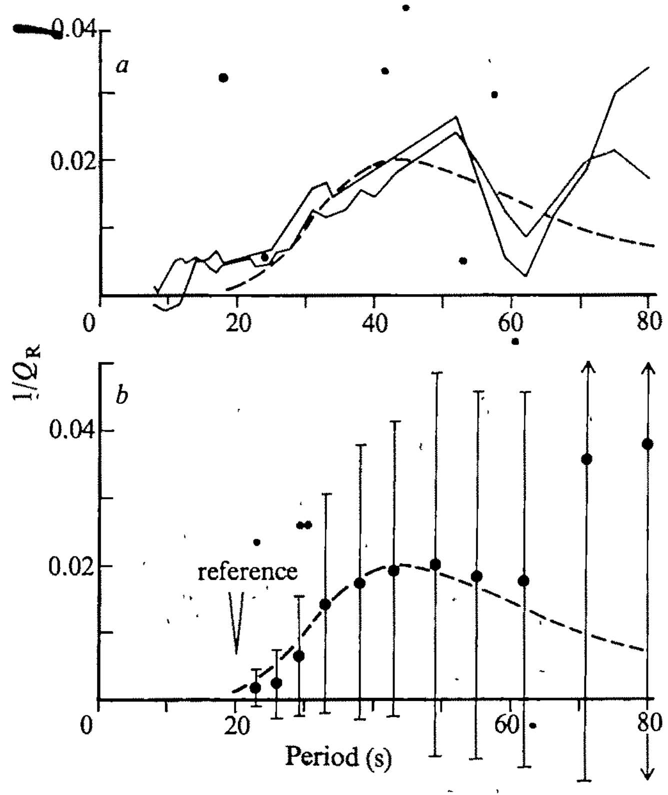
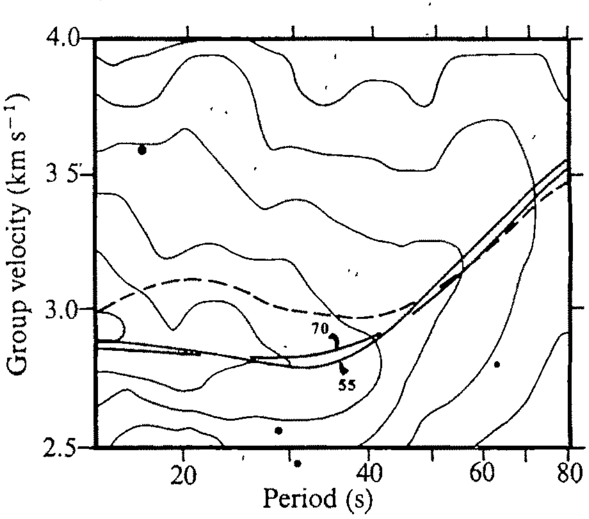


Fig. 3 Attenuation of Rayleigh waves determined from (*a*) nuclear test data and (*b*) all data combined. Bars show the s.d. in attenuation. ----, Single attenuating layer at 70-km depth. Attenuation goes to zero at 20-s period because of the normalisation performed.

strongly affected. Assuming also that there are no purely dilatational dissipations, we can find the depth to the centre of the attenuating layer. Using an Earth velocity model compatible with observations on the group velocities of Rayleigh waves and the equations for a horizontally-layered Earth[16], we find that the best-fitting single attenuating layer would be centred at 70 km below the surface. Its 'attenuation thickness' (ratio of layer thickness to Q^{-1} for shear waves) is 2 km, that is, if the layer is 10 km thick, it has a Q of 5. Such extremely low values of Q are only found in two-phase (partially melted) rocks at such high pressures. There is, also, a suggestion of a second, deeper attenuating layer in the data (Fig. 3), which is not discussed here because it is so poorly resolved.

The geological significance of the inferred partially-molten layer depends on whether it is in the crust or the mantle. From the size of the gravity anomaly[11] we can estimate that the crust is between 55 km thick (if the mantle is as hot and light as it is beneath mid-ocean ridges) and 70 km thick (assuming a normal mantle[17]). Either of these thicknesses is compatible with the group velocity data (Fig. 4) because the lower crustal shear velocity is unknown. The velocity required in the 70-km model

Fig. 4 Rayleigh wave group velocity data from 20 paths consisting of at least 50% Tibetan Plateau. Records were analysed by spectral amplitude technique[18] and the spectral amplitudes have been summed. Total power is contoured in 5-dB intervals, and the shaded area indicates a swath within 2 dB of the local maximum. ———, Models with crustal thicknesses of 55 and 70 km. ----, Model with two normal continental crusts superposed.



(3.8 km s^{-1}) is more plausible, however, than the very low velocity required in the [illegible]5-km model (3.5 km s^{-1}). Also, the volcanics at the surface are clearly of crustal origin[8], and it is natural to associate the attenuating layer with their source region.

We conclude that Tibet has a crust of about 70-km thickness, of which the lowermost part is partially molten. The inferred high temperature of the Moho is inconsistent with the crustal-underthrusting model for the formation of Tibet[9] It is suggested that the high temperatures may predate the Himalayan continental collision, and that they may have been responsible for extensive horizontal compression in Tibet after that collision, thus explaining the anomalously thick crust. At present, Tibet overlies an asthenosphere which extends into the lower crust, precluding any long term mechanical strength. This weakness at shallow depths is responsible for the uniformity of Tibetan elevations over wide areas in the centre of an orogenic zone.

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African Archaean mobile belts and granite–greenstone terrain

ARCHAEAN granite–greenstone terrains such as the west African and Rhodesian Cratons, are frequently bordered by pene-contemporaneous, long-lived, high-grade mobile belts containing granulite facies supracrustals, enderbites, granite gneisses and anorthosites. For example, in Sierra Leone[1], the granite–greenstone terrain contains low-grade supracrustal belts along NNE–SSW trends, while the discordant Kasila Group granulite facies belt trends NW–SE, and forms the coastal border of the West African Craton. A similar relationship is found between the Rhodesian and Kaapvaal Cratons, which are separated by the east–west trending Limpopo Belt[2,3] The metamorphic, structural and lithological contrasts between mobile belts and granite–greenstone terrain have been ascribed to contrasts in heat-flow regime. I attempt here to correlate field and experimental data to elucidate Archaean mobile belt–craton relationships.

Greenstone belts were probably initiated along linear 'rift-type' structural weaknesses which developed as a consequence of tensional forces within the lithosphere at right angles to their trend[4] These forces could be caused by drag from an underlying upwelling asthenosphere, as for example, in present-day axial rift zones in continental swells and mid-ocean ridges. The scale of contemporary mantl 'convection' cells and associated orogenic belts is similar to that of the mobile belts, but dwarfs the dimensions and periodicity of the Archaean greenstone belts. Experiments[5,6] show that convection leads to the formation of bimodal structural patterns; where convection cells may be primary or secondary, each having contrasting dimensions and orientations. I propose here that mobile belts represent 'super-greenstone belt' structures due to primary linear asthenospheric upwellings, whereas secondary upwellings (Richter rolls) give rise to greenstone belts, usually at a high angle to the trend of the primary structure. The initial primary upwelling divides the pre-greenstone terrain crust into two portions, separated by a zone which undergoes greenstone belt evolution that outstrips that of the relatively sluggish belts within the adjacent cratons (Fig 1) This contrast in evolution rate and scale between mobile belts and greenstone terrain is due to respective differences in heat transfer from the underlying asthenosphere

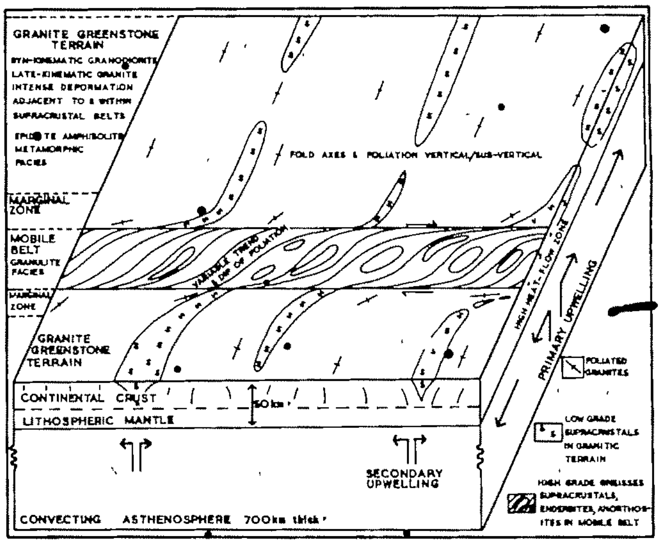
GRANITE GREENSTONE TERRAIN
SYN-KINEMATIC GRANODIORITE
LATE-KINEMATIC GRANITE
INTENSE DEFORMATION ADJACENT TO & WITHIN SUPRACRUSTAL BELTS
EPIDOTE AMPHIBOLITE METAMORPHIC FACIES
FOLD AXES & FOLIATION VERTICAL/SUB-VERTICAL
MARGINAL ZONE
MOBILE BELT
GRANULITE FACIES
VARIABLE TREND & DIP OF FOLIATION
MARGINAL ZONE
GRANITE GREENSTONE TERRAIN
HIGH HEAT-FLOW ZONE
PRIMARY UPWELLING
CONTINENTAL CRUST
50km
LITHOSPHERIC MANTLE
SECONDARY UPWELLING
CONVECTING ASTHENOSPHERE 700km thick
FOLIATED GRANITES
LOW GRADE SUPRACRUSTALS IN GRANITIC TERRAIN
HIGH GRADE GNEISSES SUPRACRUSTALS, ENDERBITES, ANORTHOSITES IN MOBILE BELT

Fig. 1 Oblique view and section of an idealised granite–greenstone–mobile belt relationship.

Heat production and transfer within the Archaean asthenosphere and mesosphere was probably more than twice its present value[4], and may have involved turbulent, rather than steady-state convective cells[5]. Elder[8] has also shown that within a convecting system there is a critical lateral distance between the mobile belt and the development of secondary linear systems. In the African Archaean cratons the greenstone belts associated with the secondary cells are frequently enveloped by the deforming marginal zones of the mobile belt[2] indicating that this critical distance was small This distance is governed by the loss of heat from the upper surface of the primary cell, which is the asthenosphere–lithosphere boundary, and the thickness of the convecting asthenosphere layer. Highly efficient loss of heat to the lithosphere could have arisen by convection within its granitic layer[7], since convective diapirs of granite frequently distort the originally linear trend of the secondary cells to produce the less ordered structure typified by the 'gregarious batholith'. Elder also noticed that natural breaks occurred within the secondary cells, which could explain the longitudinal impersistency of some supracrustal belts.

The granulite facies mobile belts are characterised by linear and dome–basin tectonic styles, where extreme deformation in the linear portions limited grain growth, and produced fine-grained basic granulites and platy quartzo-feldspathic rocks. The relative movement of the bordering cratons with respect to the mobile belt, by pure and/or simple shear, is responsible for the plastic deformation in belts and the development of thrusts at their margins. The cause of the movement may be due to small angular shifts in the orientation of the bordering cratons, as they succumb to the eddy currents in the underlying turbulently convecting asthenosphere.

The high heat flow in the mobile belt may also be the key to the close spatial and temporal relationships between granulite facies rocks and layered calcic anorthosites[4]. Anorthosites occur within and adjacent to mobile belts, and are characteristically coarse-grained, even pegmatitic, suggesting intrusion of a hydrous magma into hot crust. The restricted occurrence of layered calcic anorthosites within the Archaean[4], and of most massif-type anorthosites to mobile belts of Proterozoic age, suggests that they are formed within zones of extremely high heat flow Phanerozoic anorthosites are also restricted to zones of high heat flow where lithospheric plates have[8] or are separating[9].

Ensialic Proterozoic belts[10], and those of the Pan-African, may be caused by processes similar to those of Archaean age. The post-Archaean lithosphere, however, was relatively thicker, stronger and more sedate[11], failing to allow the formation of secondary structures, so that greenstone belts did not form contemporaneously with mobile belt activity. Phanerozoic 'plate tectonics' may represent the final examples of a decaying process now only capable of producing endosialic orogens on the margins of continental lithosphere resistant to mobile belt formation.

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Isolation of amitrole-degrading bacteria

THE herbicide, amitrole (3-amino-1,2,4-triazole) is degraded in soil within a matter of weeks under favourable conditions[1–4]. This degradation may be microbiological since it is negligible in soils sterilised by steam[1–3,5] or chemicals[2,3]. Kaufman *et al.*[6], however, consider amitrole breakdown to be primarily due to chemical processes since amitrole-degrading organisms have never been isolated from soil. They found that degradation of amitrole continued in chemically sterilised, though not in autoclaved soil. We report here the isolation of amitrole-degrading microorganisms from soil, thus strengthening the case for microbial degradation.

Tenfold serial dilutions (from 10^{-2} to 10^{-8}) of a sandy clay loam soil were prepared in each of three media: basal medium (0.03 g K_2HPO_4, 0.01 g $MgSO_4.7H_2O$ and 30 p.p.m. amitrole, pH 7 3), medium 2 (basal medium plus 3% sucrose) and medium 3 (basal medium plus 3% sucrose, 50 p.p.m. $(NH_4)_2SO_4$, 100 p.p.m. peptone and 20 p.p.m. yeast extract). The test-tubes of inoculated media were incubated at 25 °C over 6 weeks and the amitrole content in those with visible growth was measured by Sund's method[7]. Amitrole disappeared completely in medium 2 at soil dilutions of 10^{-3} to 10^{-6} (Table 1), but no degradation could be detected in any other treatment

Pure cultures were isolated from tubes showing degradation by dilution plating on to the same medium solidified with 1.5% agar. Of 36 representative isolates reinoculated into medium 2, 10 were capable of degrading amitrole; nine of these were Gram-positive rods (*Bacillus* spp. and *Corynebacterium* spp.) and one was identified as *Pseudomonas* sp.

Table 1 Degradation of amitrole by soil samples

Soil dilution	Incubation time (weeks) 1	2	3	4	5	6
10^{-2}	–	–	–	–	–	–
10^{-3}	–	–	+	+	+	+
10^{-4}	–	–	–	+	+	+
10^{-5}	–	–	–	–	–	+
10^{-6}	–	–	–	–	–	+
10^{-7}	–	–	–	–	–	–
10^{-8}	–	–	–	–	–	–

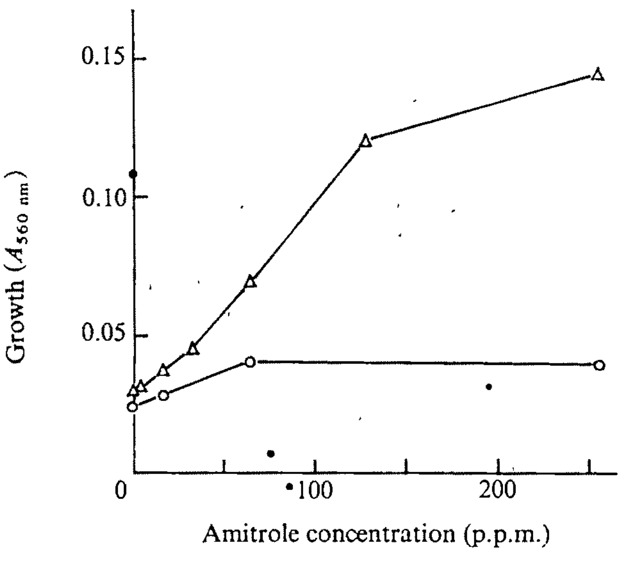
Diluted soil samples were incubated in 10-ml aliquots of medium 2 at 25 °C; –, no degradation; +, degradation.

The relationship between growth of one amitrole-degrading isolate and amitrole concentration was determined by growing the isolate in Erlenmeyer flasks of basal medium or medium 2 containing various amitrole concentrations. To prevent aerial contamination of the medium with combined nitrogen, each flask was sealed with a rubber stopper and connected with the exterior only by a U-tube containing H_2SO_4. In these conditions the isolate was unable to fix molecular nitrogen.

Growth was roughly proportional to herbicide concentration up to 256 p.p.m. amitrole in the medium with sucrose (Fig 1). There was little growth in basal medium with amitrole as the sole source of organic carbon as well as nitrogen.

The inability of other workers to isolate actively degrading organisms may have been due to their use of enrichment media with amitrole as sole source of organic carbon rather than nitrogen. It also seems that the decomposition of amitrole is inhibited in soil dilutions less than 1 in 1,000. This could be due to preferential use of other nitrogen

Fig. 1 Effect of amitrole concentration in the medium on growth of isolate Q_8 after incubation for 17 d at room temperature (20 °C average ± 6 °C). Mean of three replicates, in basal medium (○) and medium 2 (△)



sources present in soil since the presence of combined nitrogen in medium 3 prevented degradation of amitrole at any soil dilution.

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Frequency of heavy-metal resistance in bacteria from inpatients in Japan

IN many bacteria, resistance to heavy metals is associated with a plasmid[1–4]. R plasmids in *Escherichia coli* can determine resistance to several metallic ions such as mercury, cobalt and nickel[3], and a penicillinase plasmid mediating resistance to mercury, cadmium, arsenate, arsenite, lead and zinc has been observed in *Staphylococcus aureus*[1,2]. Mechanisms controlling bacterial resistances to mercury and cadmium are quite different although both are mediated by the same penicillinase plasmid[4]. Furthermore, microorganisms generally detoxify mercurial compounds metabolically by the formation of volatile mercury[5–9] or mercury mercaptides[10–12]. It is of interest that resistance to these heavy metals is mediated by the plasmids which determine resistance to antibiotics Most of these heavy metals are established or possible causes of environmental pollution, methyl mercury causes 'Minamata disease'[13,14] and cadmium causes 'Itai-Itai disease'[15,16] in Japan. The role of R plasmids in drug resistance has been widely studied[17–21], and extrachromosomal determinants are a main cause of the increase in numbers of drug-resistant bacteria. Studies of heavy metal resistance have attempted to establish a relationship between resistance to heavy metals and to drugs in the hospital environment[22,23] The factors selecting for these heavy-metal-resistant bacteria have not yet been identified. We believe that heavy-metal-resistant microorganisms do not arise by chance, but, that there must be selectional factors beyond mere drug resistance. One of these selectional factors may be environmental contamination by heavy metals. To investigate this possibility, we studied the frequency of drug and heavy-metal resistance in clinical isolates of *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *S. aureus*. We report here that the frequency of heavy-metal resistance in these strains was the same as, or higher than that of antibiotic resistance. Some R plasmids in *E. coli* and *K. pneumoniae* also carried a resistance to Hg.

Figure 1 shows the distribution curves of Hg, Cd, As and Pb resistances (from agar dilution tests), in 564 strains of *E coli*, 331 strains of *K. pneumoniae*, 787 strains of *P aeruginosa* and 515 strains of *S aureus*. The distribution of susceptibility to most of these heavy metals, against which a resistance developed, is bimodal. But in the case of a Cd resistance in *S aureus*, there were three groups of Cd sensitivity.

The frequency of a resistance to these heavy metals and to several drugs for the tested strains is shown in Table 1. The frequency of the heavy-metal-resistant strains was the same as, or higher than the frequency of drug-resistant strains. Many strains were resistant to heavy metals, but remained sensitive to the antibiotics (21.6% of *E. coli*, 27 2% of *K. pneumoniae*, 19.5% of *P. aeruginosa* and 8.0% of *S. aureus*).

We selected 348 strains of *E. coli* and *K. pneumoniae* resistant to Hg and examined the conjugal transferability of their resistance by using two recipients. One recipient, *E. coli* K12 ML1410-Nx, a nalidixic acid-resistant mutant and an As-sensitive segregant of *E. coli* K12, was used for the transfer of R plasmids in the first cycle. The other recipient, *E coli* JE17-Rif, isolated in our laboratory, is a rifampicin-resistant mutant and was used for the second cycle of transconjugation. Both recipients are sensitive to Hg, As, streptomycin (SM), tetracycline (TC), chloramphenicol (CM), kanamycin (KM) and gentamicin (GM) but resistant to Cd. Among 231 strains of Hg-resistant *E. coli*, 207 Hg-resistant R plasmids were isolated, and of these 195 were As resistant. Among 117 Hg resistant *K pneumoniae*, 110 R plasmids carrying an Hg resistance were also observed: 102 of these 110 R plasmids carried resistance to As. In *E. coli*, R plasmids with a resistance to R(Hg,As; SM,TC,CP,KM) were isolated most frequently, followed by R(Hg,As, SM,TC,CP), R(Hg,As, SM,KM), R(Hg,As, TC,CP) and R(Hg,As, SM,TC,KM), in that order. A similar result was obtained in *K. pneumoniae* The resistance patterns of R plasmids were similar to those of the resistant strains that were frequently isolated, showing that Hg resistance in these enterobacteria is mainly due to the presence of R plasmids (Table 2).

Transferable R plasmids carrying Hg resistance were present in

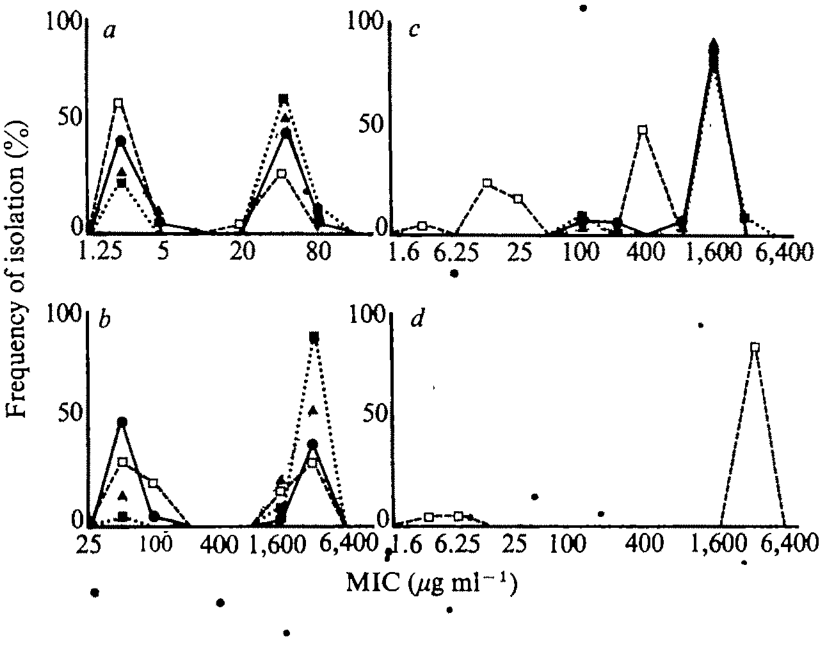

Fig. 1 The minimum inhibitory concentration (MIC) distribution curves of *a*, Hg, *b*, As, *c* Cd and *d*, Pb resistances (by agar dilution test) for 564 strains of *E. coli* (●), 331 strains of *K pneumoniae* (▲), 787 strains of *P aeruginosa* (■) and 515 strains of *S. aureus* (□). The tested strains were isolated in the Clinical Laboratory of the Jikei Hospital between 1974 and 1976 The metals, Hg, Cd, As and Pb were used as $HgCl_2$, $CdCl_2$, Na_2HAsO_4 and $Pb(CH_3COO)_2$, respectively Nutrient agar was used for the metal resistance test because $HgCl_2$ was stable in it The distribution curves of Hg, Cd and As resistances show the clear-cut distinctions between the 'sensitive' and 'resistant' populations, except that the Cd resistance in *S aureus* is divided into three groups In the case of the Pb resistance in *S. aureus*, the distribution pattern clearly revealed two peaks In *E coli*, *K. pneumoniae* and *P aeruginosa*, the distribution curves indicated that the resistance concentrations of each metal were 10 μg ml^{-1} for Hg, 400 μg ml^{-1} for Cd and 400 μg ml^{-1} for As In *S. aureus*, the resistance concentrations of Hg, As, Pb, high and intermediate Cd were 10 μg ml^{-1}, 400 μg ml^{-1}, 100 μg ml^{-1} and 10 μg ml^{-1} respectively.

Table 1 Frequency of heavy-metal resistance and drug resistance in *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* clinical isolates

		E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
		No. of resistant strains*			
Metal†	Hg	323 (57.3)	218 (65.9)	591 (75.1)	187 (36.3)
	Cd	524 (92.9)	324 (97.9)	759 (96.5)	491‡ (95.3)
	As	342 (60.6)	276 (83.4)	779 (98.9)	251 (48.7)
	Pb	—§	—	—	473 (91.8)
Drug**	PC	ND	ND	ND	454 (88.2)
	SM	377 (66.8)	223 (67.4)	405 (51.5)	144 (28.0)
	TC	344 (61.0)	170 (51.4)	125 (15.9)	171 (33.2)
	CP	315 (55.9)	163 (49.2)	324 (41.2)	113 (21.9)
	KM	186 (33.0)	122 (36.9)	404 (51.3)	119 (23.1)
	EM	ND	ND	ND	220 (42.7)
	JM	ND	ND	ND	198 (38.4)
	GM	9 (1.8)	12 (3.8)	108 (13.7)	0 (0)
Total no. of strains		564	331	787	515

*Figures in parentheses represent % of total isolates.

†Hg $HgCl_2$, Cd $CdCl_2$, As Na_2HAsO_4, Pb $Pb(CH_3COO)_2$

‡High Cd 258 (50.1%), intermediate Cd 233 (45.2%)

§We cannot detect Pb-resistant strains since the distribution pattern of Pb resistance shows only one peak

**Streptomycin(SM), tetracycline(TC), chloramphenicol(CP), kanamycin(KM) and gentamicin(GM) were at a final concentration of 12.5 μg ml^{-1} for the selection of resistant strains in *E. coli*, *K. pneumoniae* and *S. aureus*. In *P. aeruginosa*, cultures not inhibited by 200 μg of SM, TC, CP and KM per ml or 25 μg of GM per ml were regarded as resistant to each of the drugs. In *S. aureus*, resistance concentrations of penicillin(PC), erythromycin(EM) and josamycin(JM) were 1.6 IU ml^{-1} 25 μg ml^{-1} and 25 μg ml^{-1}, respectively. ND, not determined.

most (89.6% of *E. coli* and 94.0% of *K. pneumoniae*) of the Hg-resistant isolates. It should be noted that there were three R plasmids carrying only Hg and As resistance in *K. pneumoniae*. These three R plasmids were quite stable in both *E. coli* K12 ML1410-Nx and *E. coli* JE17-Rif (Table 2). This is of interest as mercury is an environmental pollutant.

Thus, frequency of a heavy-metal resistance was the same as, or higher than, that of an antibiotic resistance, and many isolates were heavy-metal resistant but drug sensitive. We isolated 317 R plasmids with an Hg resistance from Hg-resistant *E. coli* and *K. pneumoniae* (91.1% frequency). In addition, 297 R plasmids mediated As resistance.

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Table 2 Demonstration of R plasmids carrying an Hg resistance in *E. coli* and *K. pneumoniae* isolates and their patterns of resistance to As and antibiotic

Resistance pattern of R plasmid	% of *E. coli* strains with R plasmid		% of *K. pneumoniae* strains with R plasmid	
Hg, As, SM, TC, CP, KM, GM	*4*	*4 (1.9)*	*3*	*3 (2.7)*
Hg, As, SM, TC, CP, KM	*65*	*69 (33.3)*	*25*	*31 (28.1)*
Hg, As, SM, TC, CP, GM	*0*		*3*	
Hg, SM, TC, CP, KM	*4*		*3*	
Hg, As, SM, TC, CP	*63*	*81 (38.2)*	*24*	*40 (36.4)*
Hg, As, SM, TC, KM	*9*		*7*	
Hg, As, SM, TC, GM	*0*		*2*	
Hg, As, TC, CP, KM	*5*		*4*	
Hg, SM, TC, CP	*4*		*3*	
Hg, As, SM, TC	*8*	*49 (23.7)*	*9*	*28 (25.5)*
Hg, As, SM, CP	*6*		*4*	
Hg, As, SM, KM	*14*		*10*	
Hg, As, TC, CP	*12*		*2*	
Hg, As, TC, KM	*2*		*1*	
Hg, As, CP, KM	*3*		*0*	
Hg, SM, KM	*2*		*2*	
Hg, TC, CP	*2*		*0*	
Hg, As, SM	*2*	*4 (1.9)*	*2*	*5 (4.6)*
Hg, As, TC	*1*		*0*	
Hg, As, CP	*1*		*2*	
Hg, As, KM	*0*		*1*	
Hg, As	*0*	*0 (0)*	*3*	*3 (2.7)*
Total	207		110	

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Permanent segmental interchange complex in the termite *Incisitermes schwarzi*

IN a cytogenetic survey of the termites of Florida, we have found two species, *Incisitermes schwarzi* (Banks) and *Kalotermes approximatus* (Snyder) (Isoptera; Kalotermitidae), that possess segmental interchange complexes as a regular feature of their breeding system. This report is concerned with the chromosomes of *I. schwarzi*, which has the most extensive complex of reciprocal translocations so far described in any animal species. The complex involves about half of the chromosome set, and is restricted to males. The occurrence of this interchange complex may be related to the social organisation of these insects, to an inbreeding habit of reproduction, or to the evolution of a mechanism of sex determination with multiple sex chromosomes.

The diploid number for both males and females is 32; the chromosome complement contains 4 or 5 acrocentric pairs, the remainder being metacentric or submetracentric. In female meiosis there are 16 bivalents; most of them are ring-shaped, indicating the presence of a terminal chiasma in each chromosome arm (Fig. 1*a*). In male meiosis in 7 of the 8 colonies studied, there were 9 bivalents plus a ring of 14 chromosomes (Fig. 1*b*). (The males of one colony had 8 bivalents plus a ring of 16 chromosomes; see Fig. 2.) All of the males from any one colony had the same chromosome configuration. The ring of 14 seems to be composed of metacentrics and submetacentrics; the chiasmata are terminal or nearly terminal. Judging from the chromosome numbers in anaphase I groups and in second meiotic divisions, disjunction from the ring in the first meiotic division seems to be regular, half of the chromosomes moving to one pole, half to the other.

The restriction of the ring to male meiosis indicates that the male is the heterogametic sex, and that the sex chromosomes are incorporated into the ring. Although we have not yet identified the sex chromosomes in this species, this interpretation is supported by the observation that the sex ratio is approximately 1 : 1 (suggesting chromosomal determination of sex), and by the finding that in the related species *K. approximatus*, where the sex chromosomes can be more easily distinguished morphologically, they are indeed part of the ring. On this interpretation the males of *I. schwarzi* may have, in addition to one 'standard' set of autosomes and an X chromosome, six interchange autosomes and a neo-Y chromosome that evolved from an original standard set and an original Y by a series of reciprocal translocations. Alternate disjunction from the ring of 14 chromosomes formed at meiosis would ensure that the interchange autosomes would always be transmitted to the progeny along with the male-determing neo-Y chromosome (Fig. 3). An alternative possibility is that the

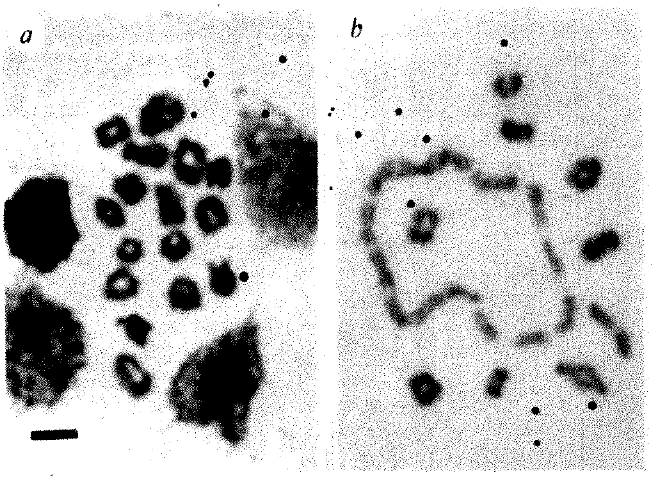
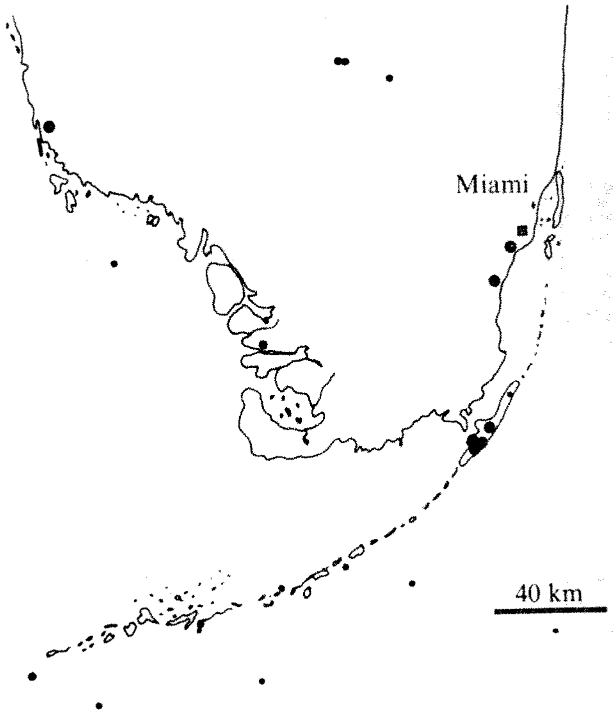


Fig. 1 Meiotic chromosomes in *I. schwarzi*. *a*, female; *b*, male. Whole termite colonies were collected from various locations in southern Florida (Fig. 2), and maintained in the laboratory on a mixture of paper towels and sawdust from termite-infested wood. Chromosome preparations were usually made within a month of collecting the colony. Gonads from winged imagoes were dissected out in 0.45% sodium citrate solution, treated for 3 min with 1.0 N HCl at about 60 °C, transferred to 45% acetic acid for 15–20 min, squashed on a slide under a coverslip, rinsed in distilled water, air-dried, and stained with Giemsa (bar, 5 μm).

Fig. 2 Locations of colonies of *I. schwarzi* studied in southern Florida. ●, Colonies with 9 bivalents and a ring of 14; ■, colony with 8 bivalents and a ring of 16.



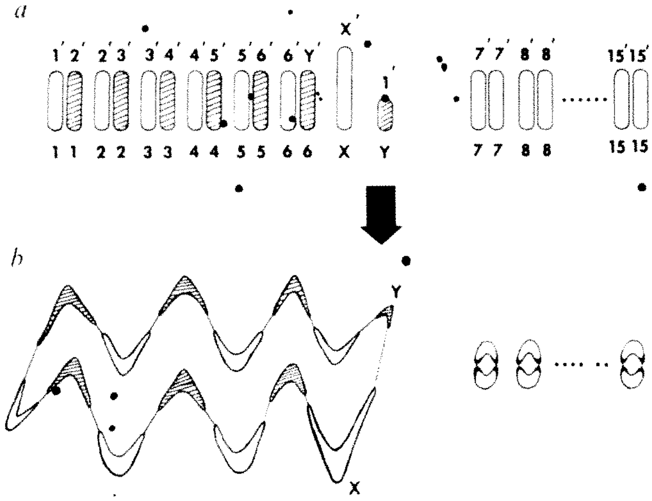

a
1′ 2′ 2′ 3′ 3′ 4′ 4′ 5′ 5′ 6′ 6′ Y′
X′
1′
7′ 7′ 8′ 8′
15′ 15′
1 1 2 2 3 3 4 4 5 5 6 6 X Y
7 7 8 8
15 15
b
Y
X

Fig. 3 Diagram illustrating the hypothetical association of a neo-Y and interchange complex (shaded) with the X and the standard set of chromosomes (unshaded) in the male. *a*, Mitotic chromosomes; chromosome ends are numbered to show an example of segmental interchanges that would lead to ring formation in meiosis (*b*). The sizes of the chromosomes are arbitrary; the X and Y chromosomes have not yet been distinguished from the autosomes nor from each other in this species.

whole interchange complex is a set of multiple, male-determining, sex chromosomes.

Such extensive reciprocal translocations, ordinarily expected to lead to chromosome imbalance in gametes and lethality in zygotes, evidently form part of a balanced system that has adaptive value for the species, as in the case of the more extensive exchanges found in the plants *Oenothera* (ref. 1) and *Isotoma* (ref. 2). It is premature to speculate in detail on the genetic and evolutionary significance of the interchange complex in *I. schwarzi*, but the following comments point up some of its implications.

An important genetic consequence of the interchange complex is that its chromosomes, and the standard set with which it is associated, behave as a single linkage group in the male; the interchange complex therefore tends to reduce the genetic variability that would otherwise result from independent assortment of the chromosomes involved. Interchange complexes similar to that found in *I. schwarzi* also occur in the males of the dioecious plant *Viscum* (refs 3, 4), the beetle *Cyrsylus volkameriae* (ref. 5), some populations of the flea *Nosopsyllus fasciatus* (ref. 6), and some species of the centipede *Otocryptops* (refs 7, 8); and possibly in some females of the freshwater copepod *Diaptomus castor* (refs 9, 10). The interchange complex in each of these cases might represent an example of the evolution of a system of multiple sex-determining chromosomes; or it may have arisen by a series of translocations between the Y chromosome of a simpler sex-determining system and the autosomes, as a means of reducing variability, or of maintaining heterozygosity, in a set of autosomal genes. The genetic effect of the interchange complex in all these organisms may be related to the reduction of crossing-over that occurs in the heterogametic sex of other organisms, notably in the males of the fruit fly *Drosophila* and in the females of the silkworm *Bombyx*.

The presence of the interchange complex might be important in the evolution of sociality in these termites, similar in its reduction of genetic variability to the systems of parthenogenesis and male haploidy that characterise the other social insects, the ants, bees and wasps[11]. The colony might provide a more uniform and stable environment, so that more genetically uniform individuals would be favoured; evolution of genetic uniformity might also be a necessary condition for the coevolution of the intricate hormonal and behavioural interactions that take place among the individual members of the colony. (Note, however, that while *I. schwarzi* and *K. approximatus* both have extensive interchange complexes, other species of termites—*Termopsis angusticollis* (ref. 12), *Reticulitermes flavipes* (ref. 13), *Odontotermes redemanni* (ref. 14) and *Neotermes jouteli* (unpublished observations)—do not.)

The presence of an interchange complex in *I. schwarzi* may be the evolutionary consequence of inbreeding. The interchange complexes of *Oenothera* and *Isotoma* are associated with a self-pollinating habit of reproduction[1,2]; Darlington and La Cour[15] obtained evidence in the plant *Campanula* that selfing and inbreeding favour, at least transiently, the maintenance of interchange complexes; and Lewis and John[16] found interchange complexes in some isolated populations of roaches. In termite species in general, the reproductives that appear in the mature colony, at the time of swarming, are usually siblings. Because they are weak fliers and may not travel far from the original colony, and since in any case different colonies in an area may swarm at different times[17], new colonies in general are probably founded by two siblings from the same original colony, rather than by a male and a female from different colonies. These termites therefore may be highly inbred, and thus might provide another example of the correlation between inbreeding on the one hand and the evolution and maintenance of structural heterozygosity on the other.

Note added in proof: We have recently found a third termite species that has an interchange complex in male meiosis: *Neotermes castaneus* (Burmeister) (Kalotermitidate).

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Parallel variation at an enzyme locus in sibling species of field crickets

ELECTROPHORETIC surveys of levels of heterozygosity in animal species have established that populations often contain large amounts of genetic variation at loci coding for soluble proteins[1–3]. This has led to an extended debate about the causes of such variation. In particular, two opposing views have emerged, one emphasising the importance of balancing selection in maintaining the protein variation (selectionist) and the other emphasising the importance of stochastic processes (neutralist)[4–6]. Here I examine spatial patterns of variation at a single enzyme locus in two closely related insect species. I argue that since these sympatric species not only have similar mean frequencies of particular electrophoretic variants, but also exhibit a common pattern of geographic variation, parallel selection provides the only satisfactory explanation.

As attempts to distinguish between the neutralist and selectionist hypotheses, studies of spatial patterns of variation have often proved to be ambiguous. In the absence of independent and accurate estimates of mutation rate, effective population size, gene flow or (in the case of interspecific comparisons) time since divergence, both neutralist and selectionist arguments are sufficiently flexible to explain most observed patterns of variation. Moreover, since allelic variants can have identical electrophoretic mobility, electrophoretically detectable variation may represent only a fraction of the total variation[7-10]. A single electrophoretic mobility class ('electromorph'[11]) may include many alleles with common mobility. Indeed, heterogeneity within single mobility classes has been demonstrated in several enzyme systems[10,12-14]. Recognising this possibility, recent models which consider the behaviour of slightly deleterious alleles suggest that similarity of electromorph frequencies among apparently isolated populations or species may reflect independent approximations of the same mutation–selection equilibria rather than the effects of balancing selection[11,15,16]. But the observation that electromorph frequencies vary in parallel in two sympatric species can only be explained by the latter hypothesis. Previous studies of enzyme variation in mussels[17] and fish[18] have documented possible examples of parallel response to environmental factors.

Gryllus veletis and *Gryllus pennsylvanicus* are sibling species of field crickets (Orthoptera, Gryllidae) found together in southern Canada, throughout the north-eastern United States, and south in the Appalachian Mountains to North Carolina[19-22]. They are exceedingly similar morphologically, have identical calling songs and are found in identical habitats[19,21]. They can be distinguished only on the basis of differences in their life cycles[20-25]. *G. veletis* undergoes diapause as a late instar nymph and matures in May and June. *G. pennsylvanicus* undergoes diapause in the egg stage and adults appear in late summer.

Table 1 Mean PGI electromorph frequencies in *G. veletis* and *G. pennsylvanicus*

Electromorph	*G. veletis*	*G. pennsylvanicus*
PGI^{160}	0.001	—
PGI^{135}	0.040	0.105
PGI^{125}	0.046	0.019
PGI^{100}	0.804	0.797
PGI^{65}	0.097	0 079
PGI^{25}	0.010	—
n	40	52

Each value represents the unweighted mean of electromorph frequencies in 11 populations from the eastern United States. $\bar{n}$ is the average sample size (number of individuals). Localities are described in Fig. 1. The most common electromorph is designated 100 and other electromorphs are given numbers corresponding to their relative mobilities. Crickets were frozen soon after collection and stored at −80 °C. They were thawed immediately before electrophoresis, the head and wings were removed and soluble proteins were extracted by homogenising legs, thorax and abdomen in 0.4–0.6 ml of 0.1 M Tris, 0.001 M EDTA, 5×10^{-5} M NADP, *p*H 7.0. Electrophoresis of phosphoglucose isomerase was on 12% starch gels using the discontinuous LiOH buffer of Selander *et al.*[26].

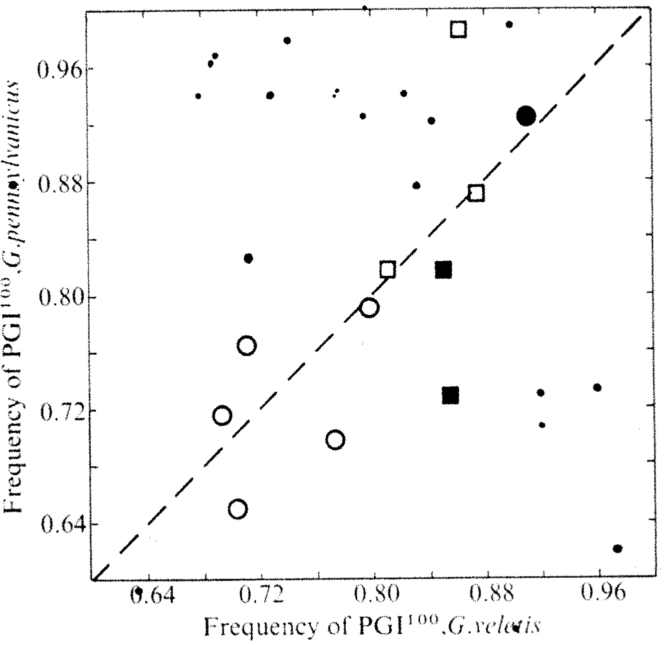

Fig. 1 Frequencies of PGI^{100} in sympatric populations of *G. veletis* and *G. pennsylvanicus*. Each point represents a single collecting locality. Some localities were sampled in 1974, others in 1975. In no case, however, have data from more than 1 yr been combined. Along the dashed line electromorph frequencies are equal in the two species. $r_s = 0.82$, $P < 0.01$. r_s is the Spearman rank correlation coefficient. Localities: ○, Tompkins Co., New York; ●, Litchfield Co., Connecticut; □, Augusta Co. and Rockbridge Co., Virginia; ■, Alleghany Co., North Carolina.

Using starch gel electrophoresis combined with histochemical staining, I examined patterns of variation at 17 loci in *G. veletis* and *G. pennsylvanicus* for evidence of parallel variation in electromorph frequency. Of these 17 loci, nine are essentially monomorphic in both species. Of the polymorphic loci, phosphoglucose isomerase (PGI) is unique in having nearly identical mean electromorph frequencies in the two species (Table 1). Analysis of PGI phenotypes in parents and offspring from single-pair matings confirm that the observed variation is genetic. Zygotic frequencies among the progeny are always consistent with segregation patterns predicted from the presumed parental genotypes (Table 2).

To test for the possibility of parallel variation at the PGI locus, I plotted the frequency of the most common electromorph, PGI^{100}, in eleven populations of *G. pennsylvanicus* against the frequency of the same electromorph in sympatric populations of *G. veletis* (Fig. 1). Samples were taken in the late spring (*G. veletis*) or late summer (*G. pennsylvanicus*) of 1974 and 1975. For each data point late instar nymphs or adults of both species were collected from identical localities. There is an obvious correlation in electromorph frequency ($r_s = 0.82$, $P < 0.01$, Spearman rank correlation coefficient). Moreover, the data points fall

Table 2 Genetic crosses of phosphoglucose isomerase variants in *G. veletis* and *G. pennsylvanicus*

			Observed (expected*) No. of each phenotype among progeny					
Cross	Parental phenotypes	Species	100/160	100/125	100	65/100	25/100	P†
1	100 × 100	p	—	—	7(7)	—	—	—
2	100 × 100	v	—	—	18(18)	—	—	—
3	100/160 × 100	v	23(17)	—	11(17)	—	—	$0.1 > P > 0.05$
4	100/125 × 100	v	—	8(8)	8(8)	—	—	> 0.9
5	100/125 × 100	v	—	16(15)	14(15)	—	—	$0.9 > P > 0.5$
6	65/100 × 100	p	—	—	17(17)	17(17)	—	> 0.9
7	100 × 65/100	v	—	—	11(15)	19(15)	—	$0.5 > P > 0.1$
8	100 × 25/100	v	—	—	10(9)	—	8(9)	$0.9 > P > 0.5$
9	65 × 100	p	—	—	—	15(15)	—	—
10	65 × 100	p	—	—	—	14(14)	—	—

*Expected assuming normal Mendelian segregation with no dominance.
†χ^2 probability. χ^2 calculated by using correction for small sample size.

close to the line of equal frequency; that is, the frequency of PGI^{100} tends to be the same in sympatric populations of the two species in spite of significant differences in electromorph frequency between allopatric populations of the same species. A plot of PGI^{65} frequencies in the same sympatric populations of *G. veletis* and *G. pennsylvanicus* also yields a significant correlation ($r_s = 0.66$, $P < 0.05$).

All the collecting localities (including those within the same county) are thought to represent distinct populations. Both cricket species are predominantly short-winged (more than 99% in the populations examined), and dispersal by flight is rare. Evidence from mark-recapture studies indicates that adults do not disperse very far on foot, even within areas of favourable habitat. Moreover, among populations of *G. veletis* and *G. pennsylvanicus* from Tompkins County, New York, three of five polymorphic loci in each species exhibit significant heterogeneity in electromorph frequency (my unpublished results).

Hybridisation and introgression between the two cricket species cannot be invoked to explain the data. In spite of repeated attempts, hybrid progeny have never been produced in laboratory matings[20–22,27]. Furthermore, I have found that natural populations of *G. veletis* and *G. pennsylvanicus* are fixed for different electromorphs at the phosphoglucomutase-1 locus. No heterozygotes have been detected among 266 *G. veletis* and 206 *G. pennsylvanicus* examined.

The environmental factors responsible for the observed pattern of variation have not been identified. The correlation in electromorph frequencies, however, is in large part due to parallel changes in mean electromorph frequency between crickets from different geographic areas (Fig. 1). *G. veletis* and *G. pennsylvanicus* from Tompkins County, New York, have significantly lower frequencies of PGI^{100} than populations from the mountains of Virginia. Crickets from Litchfield County, Connecticut, have distinctly higher frequencies of that electromorph. Superimposed on the regional pattern is considerable local variation. Although possibly due to selection, local variation may also result from random differentiation in relatively small populations with low interpopulation dispersal rates.

Two important questions remain unanswered. First, do the PGI^{100} and PGI^{65} electromorphs represent single allelic variants or are they heterogeneous assemblages of variants with common electrophoretic mobility? Second, does the PGI locus itself respond to environmental selection or is the observed pattern of variation the result of selection at a closely linked locus? If PGI^{100} and PGI^{65} represent classes of alleles with common mobility, then parallel variation may imply greater functional similarity of allozymes within a single mobility class than between classes. On the other hand, if PGI^{100} and PGI^{65} each represent a single allele, then linkage alone can explain the parallel variation if recombination frequencies between the PGI locus and the locus under selection are very small (because disequilibrium must have persisted in both species since their divergence). Answers to these questions must await detailed structural and functional characterisation of individual electromorphs.

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Possible role for acetylcholine in aggregation centre spacing in *Polysphondylium violaceum*

WHILE ridding my laboratory of an invasion of flies, I tested the toxicity of an organophosphate insecticide, 2,2-dichlorovinyldimethylphosphate (DDVP), against the growth and development of *Polysphondylium violaceum* strain PvD 7206. Plate cultures of the slime mould exposed to the vapours of a pest strip containing the insecticide seemed to grow and develop normally in most respects, but showed a startling increase in the number of aggregation centres and sorocarps formed. I report here the results of preliminary investigations on the basis for this phenomenon.

A major biochemical effect of the organophosphates is inhibition of cholinesterase and/or other serine-esterases by phosphorylation of serine at the active site. DDVP seems to be a direct inhibitor, acting without prior metabolic conversion by the organism[2]. The effect of exposure to the pesticide strip could be most nearly duplicated by exposing postvegetative cells deposited on membrane filters to filter pads saturated with a solution of 3×10^{-1} M DDVP (Fig. 1*a*, *b*). Concentrations below 1×10^{-4} M were not effective; concentrations above 5×10^{-3} M inhibited aggregation. It should be noted that the exposure conditions are not precisely equivalent. Cells exposed to the pesticide strip are under continuous assault from the vapours, whereas DDVP on filters is at maximum concentration only at the beginning of the exposure period. The compound is volatile and may evaporate from the filter surface. It is also subject to attack by phosphatases[2] and may be actively degraded by the slime mould.

The choice of PvD 7206 (ref. 1) for the initial assay of the DDVP effect proved to be fortunate. In dense plate cultures this strain aggregates with some reluctance; confluent cultures form extensive tortuous streams, but may produce only a few decisive centres and large branched sorocarps. Exposure to DDVP vapours increases the number of centres produced in these conditions nearly 100-fold. Other stocks of *P. violaceum*, for example, PvD 7209 (ref. 1), normally aggregate avidly, producing hundreds of sorocarps, and the effect of DDVP is less spectacular. In populations of postvegetative amoebae allowed to develop on filters, there is little difference in the response of the two strains to DDVP; the number of centres is increased 2- to 4-fold in either case. The low frequency of centre emergence seen in confluent cultures of PvD 7206 may indicate that this strain is unusually sensitive to the centre-

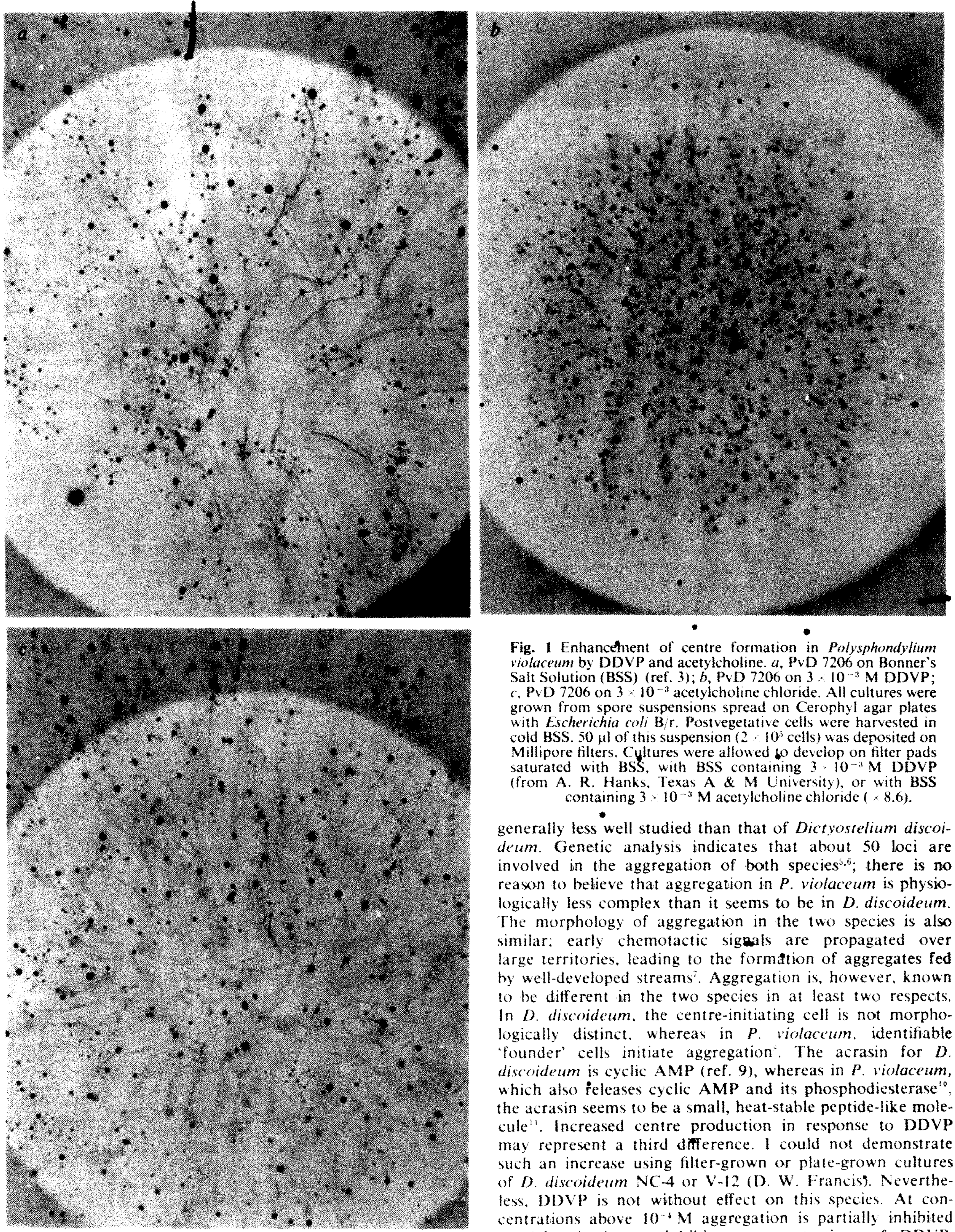

Fig. 1 Enhancement of centre formation in *Polysphondylium violaceum* by DDVP and acetylcholine. *a*, PvD 7206 on Bonner's Salt Solution (BSS) (ref. 3); *b*, PvD 7206 on 3×10^{-3} M DDVP; *c*, PvD 7206 on 3×10^{-3} acetylcholine chloride. All cultures were grown from spore suspensions spread on Cerophyl agar plates with *Escherichia coli* B/r. Postvegetative cells were harvested in cold BSS. 50 μl of this suspension (2×10^5 cells) was deposited on Millipore filters. Cultures were allowed to develop on filter pads saturated with BSS, with BSS containing 3×10^{-3} M DDVP (from A. R. Hanks, Texas A & M University), or with BSS containing 3×10^{-3} M acetylcholine chloride (× 8.6).

inhibiting mechanism involved in spacing in this species. Inhibition of centre formation by a diffusible substance emanating from previously established centres has been demonstrated in *P. violaceum*[4], but the inhibitor has not been identified.

The physiology of aggregation in *P. violaceum* is generally less well studied than that of *Dictyostelium discoideum*. Genetic analysis indicates that about 50 loci are involved in the aggregation of both species[5,6]; there is no reason to believe that aggregation in *P. violaceum* is physiologically less complex than it seems to be in *D. discoideum*. The morphology of aggregation in the two species is also similar; early chemotactic signals are propagated over large territories, leading to the formation of aggregates fed by well-developed streams[7]. Aggregation is, however, known to be different in the two species in at least two respects. In *D. discoideum*, the centre-initiating cell is not morphologically distinct, whereas in *P. violaceum*, identifiable 'founder' cells initiate aggregation[8]. The acrasin for *D. discoideum* is cyclic AMP (ref. 9), whereas in *P. violaceum*, which also releases cyclic AMP and its phosphodiesterase[10], the acrasin seems to be a small, heat-stable peptide-like molecule[11]. Increased centre production in response to DDVP may represent a third difference. I could not demonstrate such an increase using filter-grown or plate-grown cultures of *D. discoideum* NC-4 or V-12 (D. W. Francis). Nevertheless, DDVP is not without effect on this species. At concentrations above 10^{-4} M aggregation is partially inhibited or delayed. At non-inhibitory concentrations of DDVP, centre and slug formation seem normal, but at culmination the slug tip regresses, the sorogens become club-shaped and may arrest without further differentiation or produce sorocarps with relatively short stalks. Slug migration is also suppressed. Hanks (personal communication) has noted that another organophosphate, malathion, both accelerates aggregation and increases the number of centres formed in submerged populations of *D. discoideum* Ax-2. Whether this represents a response similar to that of *P. violaceum* to DDVP has not been established. It may be a unique consequence of the use of malathion, the difference in assay conditions, or the use of Ax-2; other idiosyncratic responses of the axenic strains have been noted[12]. Some organophosphates may, by acting as phosphate donors competitive with ATP, intervene in the membrane phosphorylation that precedes and has been reported to accelerate aggregation in *D. discoideum* (ref. 13).

The increased centre production by *P. violaceum* in the presence of DDVP suggests that cholinesterase or another serine-esterase is instrumental in determining the size of the aggregation territory in this species. The possible existence of a cholinergic communication system in this cellular slime mould is appealing in view of the role of acetylcholine in synaptic transmission, and the suggestion of McMahon[14] that neurotransmitters may function as developmental regulators. Preliminary evidence that the DDVP effect is at least in part a consequence of interference with a cholinergic mechanism is summarised in Table 1. Organophosphate inhibition of cholinesterase might be expected to facilitate an increase in the concentration of acetylcholine. Postvegetative cells of *P. violaceum* 7206 exposed to exogenous acetylcholine (3×10^{-3} M) showed an increase in centre production similar to that seen with DDVP. Culmination of these centres may, however, be incomplete, as the number of sorocarps produced seems to be intermediate (Fig. 1*c*); acetylcholine-treated populations produce more sorocarps than control populations, but fewer sorocarps than DDVP-treated populations. Atropine, an acetylcholine antagonist, reduces or inhibits centre formation. Eserine, a known inhibitor of cholinesterase, also stimulates centre formation, although its effect is less striking than that of DDVP. The difference may reside in their mode of action; eserine binds reversibly to cholinesterase, while the dimethyl phosphate of DDVP is covalently attached to the enzyme[2]. DDVP may also have effects other than those related to cholinesterase inhibition. These observations suggest that acetylcholine reduces territory size in *P. violaceum* and that cholinesterase increases it. Further speculation about the mechanism of this activity and its relationship to cyclic nucleotide metabolism and the ionic fluxes of aggregation should be reserved until the elements of a cholinergic system are isolated and unequivocally identified. *P. violaceum* synthesises several DDVP-sensitive enzymes with esterase activity (Clark, McCoy, and Whitmore, unpublished observations); an effort to associate one or more of these enzymes with the centre-increasing effect of DDVP, and to identify a cholinesterase among them, has been initiated.

Table 1 Effect of various additives on centre production of *P. violaceum*

Additive	Relative no. of centres formed
BSS	1.0
DDVP	
3×10^{-3} M	4.0
1×10^{-3} M	2.5
Acetylcholine	
3×10^{-3} M	3.5
1×10^{-3} M	1.1
Atropine	
3×10^{-3} M	0
1×10^{-3} M	0.6
Eserine	
1×10^{-3} M	2.6
1×10^{-4} M	1.6

Filter cultures were prepared as indicated in Fig. 1, except that some experiments were done using drop sizes at 10 or 20 µl for easier centre counting. Number of centres was determined by counting the average number of centres formed on three filters. Data from several independent experiments were combined for comparison by setting all figures relative to an arbitrary control (BSS) value of 1.0. The number of centres produced by control cultures averaged 17 for 10-µl drops and 90 for 50-µl drops. Variation for any set of testing conditions was 20% or less.

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Frequency of mating-type switching in homothallic fission yeast

THE genes determining mating type in the fission yeast *Schizosaccharomyces pombe* are of particular interest in the general study of the genetic control of differentiation. The genetic segments concerned, *mat1*^{M} and *mat2*^{P}, specifying mating type (−) and (+), respectively[1], are present, closely linked, in homothallic strains[2], but on any one chromatid (that is in the *cis* position) they are only expressed in a mutually exclusive manner[3]. The segments of the mating-type locus, *mat1*^{M} and *mat2*^{P}, govern the transition from vegetative growth to sexual reproduction. They act in a complementary fashion—one functional *M* segment and one functional *P* segment are always required to initiate both conjugation (usually among haploid cells) and meiosis (leading to sporulation in either zygotes or diploid cells), the prominent events in differentiation in *S. pombe*. A mating-type mutant defective in meiosis but not in conjugation (*mat1*^{M} *mat2*^{Pm}) when crossed with wild type, produces sporulating and non-sporulating zygotes (Fig. 1). These two classes indicate that in individual cells of either parent strain only one or the other mating-type segment is active[3]. Here I describe a single-cell evaluation of this cross, and show (also by single-cell techniques) that the frequency of mating-type switching in haploid and diploid strains is approximately random. A favoured mechanism for this type of switching is the inversion of a promoter element between the structural genes, the direction of the promoter determining which mating-type segment is switched on.

Cells of *S. pombe* are usually haploid during vegetative growth. It is only when the culture medium becomes exhausted, particularly of nitrogen, that diploid zygotes are formed by pairwise cell fusion. If left undisturbed, these zygotes undergo meiosis and sporulation, thereby immediately regenerating the haploid state. Yet it is possible to induce diploid division in a small fraction of wild-type zygotes or very efficiently in certain mutants[4]. When Leupold introduced *S. pombe* into basic genetics (see ref. 2), his strain already consisted of a mixture of heterothallic clones, in which either mating type ((+) or (−)) is heritably stable or nearly so, and of homothallic ones, in which conjugation is possible even among sister cells. Heritable changes of mating type occur in various directions[1-3], usually of the order of 10^{-4} per cell division;

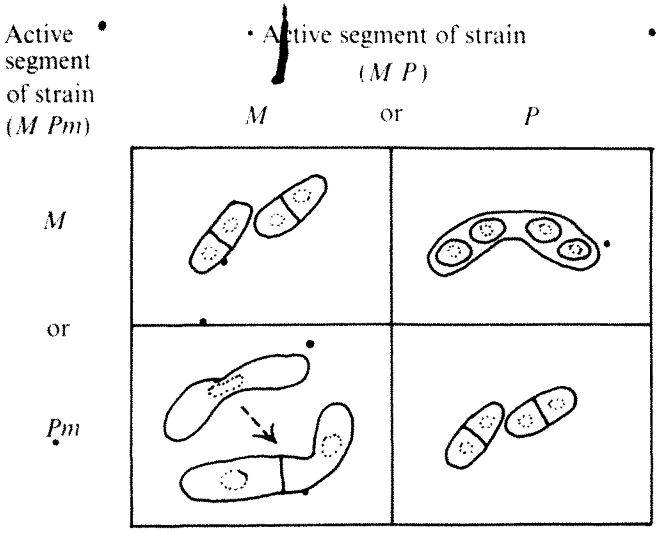
Active segment of strain (M Pm)
Active segment of strain (M P)
M
or
P
M
or
Pm

Fig. 1 Zygote classes of cross (*M Pm*) × (*M P*). On the assumption that cells of either parent strain can only activate one *mat* segment or the other, the possible four pair-wise combinations are indicated by this scheme. Two cells expressing the same mating type do not interact and, at best, divide again mitotically. Two cells expressing opposite mating types can either sporulate, if the (+) cell is wild type, or not, if the (+) cell is mutant for the *Pm* allele. Since the genetic block of this mutation is effective before commitment to meiosis[4] and before premeiotic DNA replication[10], most zygotes of the latter class can be induced to resume diploid division by a transfer to growth medium.

this is more frequent than ordinary mutations, though well below the rapid switching reported here.

In the cross of (*M Pm*) × (*M P*), illustrated in Fig. 1, both parent strains are homothallic and can produce zygotes by selfing, in addition to the really informative zygotes of hybrid descent. Colonies originating from the various classes of zygote can be distinguished by the expression of marker genes (two *ade6* alleles in this cross) (Table 1). Evidently selfing (I, II) was still preferred over crossing (III, IV); but the hybrid colonies (III compared with IV) are compatible with a 1:1 distribution with regard to the two classes of zygote indicated in Fig. 1. A few non-sporulating zygotes in fact give rise to segregating haploid colonies rather than to diploid ones, leading to incidental misclassification. Some additional uncertainty is introduced by the unclassified number of zygotes (V) that failed to form colonies. The colonies produced by class III zygotes contain sporulating and non-sporulating diploid cells. This aspect is discussed below.

The next point of interest is the frequency with which cells can alternate their choice as to which mating-type segment is to be expressed. Unfortunately, clear-cut tests for both (+) and (−) mating-type activities of single cells, such as have been worked out for budding yeast[5], are not yet feasible in *S. pombe*.

Table 1 Colonies from zygotes of cross (*M Pm*) *ade6-M216* × (*M P*) *ade6-M210*

	Colour	Colonies	Interpretation
I	Light red	109	diploid, homozygous for *M216*; selfing of (*M Pm*)
II	Dark red	68	haploid, *M210*; selfing of (*M P*)
III	White	28	diploid, heterozygous *M-216*/*M210*; cross, diploidised zygotes
IV	Streak-sectored, light/dark red	37	haploid, *M216* and *M210*; cross, sporulated zygotes
V	Zygotes that failed to grow	76	?

Cells of the strains (*M Pm*) *ade6-M216* and (*M P*) *ade6-M210* were crossed by the shift method[10] (transfer to liquid medium devoid of a nitrogen source). After agglutination had just started, the culture was briefly sonicated to reduce the chance of selfing in either parent strain. Two hours later, samples were streaked on agar slabs, and individual zygotes were isolated by micromanipulation. The slabs were then transferred to YEA plates (yeast extract agar, unsupplemented with adenine) and incubated for colony production. On this medium, both *ade6* alleles used in this cross can be distinguished by different intensities of a pink pigment. No pigment is formed in heterozygous diploid colonies, owing to allelic complementation[11].

In the experiment considered here, a haploid, homothallic strain has been used that is wild type at its mating-type locus (*M P*) but mutant at two other genes, *fus1* and *cdc2*. Conjugation is initiated as usual, but cell fusion is prevented by the *fus1* mutation[4]. The *cdc2* marker is a temperature-sensitive cell cycle mutation blocked in nuclear division[6].

The rationale was as follows. Haploid cells affected by *fus1* could at best form 'prezygotes', pairs of cells recognisable by their attached conjugation tubes. The asci observed after high-temperature exposure could only have arisen by endo-diploidisation (provoked by *cdc2*). The sister chromatids emerging from the previous replication are distributed to sister cells if division is possible, or remain within the same cell after endo-diploidisation. Since different mating-type segments must be activated on sister chromatids in order to produce a pair of prezygotic cells after one division, or a single ascus without division, these events are suitable indicators of mating-type switching.

Results are given in Table 2. If the frequency of switching were high enough for one mating-type segment or the other to be activated at random on any one chromatid, just half the uninucleate cells plated to sporulation medium should develop to a single ascus or a pair of prezygotic cells. The observed frequencies reach about half this expectation, and may actually be closer to randomness if the efficiency of conjugation induction or of endo-diploidisation is less than 100%.

Table 2 Oppositely activated mating-type segments on sister chromatids of strain (*M P*) *cdc2 fus1*

	Hours at 36 °C		
	0–2.5	3.5–5	20
Total	451	184	56
1n	322	131	40
2pz	68	(5)	—
1a	(1)	34	—
Fraction	2pz/1n =0.21	1a/1n =0.26	

1n, uninucleated cells at plating, as calculated from the fraction of single cells in the last column;

2pz, one pair of prezygotic cells produced by the first division;

1a, single ascus, produced without division.

Haploid cells of the strain (*M P*) *fus1 cdc2* were cultured in liquid sporulation medium supplemented by a nitrogen source[5]. Cells were then plated on agar medium of the same composition but lacking the nitrogen source. Only one or two residual divisions are usually observed on this medium before induction of conjugation and/or meiosis. The plates were incubated at 36 °C for different lengths of time and shifted back to 30 °C. About 20 h after plating, all plates were inspected microscopically, and the different combinations of prezygotic cells, asci, or neutral cells that had been formed after one or two residual divisions were counted. The most informative configurations (2pz and 1a) are represented here. One plate (last column) was incubated at 36 °C throughout to calculate the fraction of plated cells that had been arrested without division at the non-permissive temperature (corresponding to the uninuclear fraction at the time of plating).

The third experiment was designed to estimate frequencies of switching in diploid cells heterozygous for (*M Pm*)/(*M P*), which had been derived from the first cross (Table 1, class III). This test was performed as a pedigree analysis of cells on a growth-limiting and sporulation-inducing medium. In principle, each newly separated cell had three choices as depicted in Fig. 2. The rationale was that the different combinations of activated *mat* segments, which are indicated for separate haploid cells by Fig. 1, are now carried by individual diploid cells. A cell could develop into an ascus, provided that mating-type segments were properly activated in the wild-type combination, or develop the 'premeiotic' phenotype peculiar to the *Pm* mutation; or else it could divide again mitotically. The first two possibilities represent the alternative choices of complementary mating-type activation. The pattern by which these configurations are distributed through the

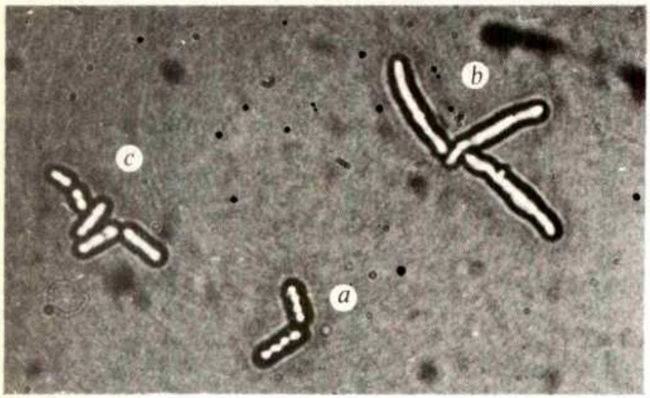

Fig. 2 Three choices of heterozygous (*M Pm*)/(*M P*) cells. Diploid cells of a heterozygous (*M Pm*)/(*M P*) strain were plated to sporulation medium, and observed after being incubated overnight. The progenies of three individual cells are depicted in this illustrative, though highly selected view, in which descendants of any one cell happened to be identical. *a*, Two asci have developed after one division; *b*, three 'premeiotic' cells have resulted from two divisions; *c*, five still vegetative-looking cells have been formed by four divisions. These possibilities were used to infer the states of activation for the different *mat* alleles during pedigree analysis. The 'premeiotic' phenotype, attributable to the *Pm* mutation, is equivalent to the aberrant elongation generally observed when DNA replication is selectively inhibited.

pedigrees should reveal for how many divisions a given combination is propagated, or how frequently the cells can switch.

Data of this analysis are given in Table 3. In *a*, single cells are evaluated with respect to the three choices listed above. The observed distribution is somewhat skewed in favour of dividing cells and premeiotic cells. In *b*, cell pairs (sister cells) are evaluated, and compared with the expectation based on a random choice after each division and biased by the skewed distribution seen in *a*. Evidently, even pairs (cc, pp, aa) are favoured over odd pairs (pc, ac, pa). Hence, a low degree of heritability has to be assumed for the different combinations of activated *mat* segments. On the other hand, the 'pa' pairs (an ascus formed next to a premeiotic cell) deviate relatively little from the expected value.

A random-choice distribution corresponds to an average frequency of 0.5 switches per chromatid and division. If the actual frequency were half that value, the expected value for a double event (changes on both chromatids), such as the formation of a 'pa' pair, should have dropped to a quarter: from 75 to 19. This value is considerably lower than the observed 58 pairs of this kind.

Randomness as to which *mat* segment is activated on any one chromatid is therefore fairly well approximated in both haploid and diploid cells of homothallic *S. pombe* strains. The *cis*-acting functional exclusion of either mating-type segment by the other can best be explained by intra-strand recombinational models that allow for rearrangements of structural genes relative to their controlling signals. Since, in *S. pombe* at least, the respective silent segment of the mating-type locus is still located at its original position on the chromosomal map, a flip-flop model by means of an invertible promoter element between the structural genes of the $mat1^{M}$ and $mat2^{P}$ segments is favoured for this yeast[7]: the relative direction of the promoter element then determines the mating-type segment to be activated in conditions for conjugation. Such a scheme has the advantage that a variety of genetic instabilities, all localised at the mating-type locus[1-3], can be attributed to a common mechanism of site-specific recombination. There are, of course, other explanations for a random-choice mechanism, but mutants have been described[8] that seem to reduce the rates of switching, so that sizeable clones of one mating type or the other are produced, which can best be explained by reversible alterations at the level of the gene.

Table 3 Pedigrees of diploid (*M Pm*)/(*M P*) cells

a	c	p	a	Total		za
Observed	1322	488	328	2138		(32)
Fraction	0.62	0.23	0.15			
b, pairs	cc	pp	aa	pc	ac	pa
Observed	494	136	47	158	176	58
Expected	408	56	25	302	203	75

c, Cell divided again.
p, Premeiotic cell.
a, 'Azygotic' ascus (sporulated cell with haploid spores).
za, 'Zygotic' ascus (sporulated zygote with diploid spores).

Diploid (*M Pm*)/(*M P*) cells were cultured and streaked to agar slabs as indicated below Table 2. Contrary to the previous experiment, however, more than two residual divisions were possible this time, owing to some impurities in a new batch of agar. Pedigrees were started by isolating cells that had just divided. For several generations, sister cells were separated by micromanipulation. The slabs were then incubated overnight and evaluated microscopically. Since conspicuous clustering of any sort was not noticed in these pedigrees, only pairs of sister cells are considered here, and the data are pooled for the first four rounds of cell division. Occasionally, 'zygotic' asci had been formed by inseparable sister cells; but these have not been considered further, since their interpretation is ambiguous.

The independent control of mating-type switching on both chromosomes of diploid *Schizosaccharomyces* cells is at variance with homothallism in *Saccharomyces*, where switching ceases after conjugation, and the resulting diploid cells stably transmit a once assumed *a*/α configuration[9] (*a* and α are the mating types of that yeast). This discrepancy seems to be related to the different life cycles of both yeasts. Most budding yeasts have evolved to a stable diploid growth phase by means of conjugating under growth conditions and zygotic budding, whereas fission yeasts do not usually grow as diploids, since conjugation is confined to starvation and is followed by zygotic reduction and sporulation. Whereas the latter condition does not require a special stabilisation for the diploid state, continued mating-type switching is incompatible with the life cycle of a budding yeast, since it would inevitably lead to excessive polyploidisation.

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Rapid, chloramphenicol-resistant, activation of membrane electron transport on germination of *Bacillus* spores

KEILIN[1] observed that the life cycle of diverse organisms includes a stage when life seems to be arrested, which he termed the cryptobiotic state. As an example of this, the mature bacterial spore can remain dormant with no detectable metabolism for long periods and then on exposure to germinants, rapidly germinate and outgrow in a synchronous manner, to yield a vegetative cell. We have been particularly interested in the composition and activities of the membrane-bound electron transport chain in sporulating *Bacillus megaterium* KM (ref. 2) and in the unique reversed orientation which characterises the membranes surrounding the developing spore[3]. This report describes the rapid changes observed in the membrane of *Bacillus megaterium* KM spores during germination and outgrowth. A rapid chloramphenicol-resistant, activation of membrane NADH oxidase activity occurred within 5–10 min of germination. Membrane-bound DL-α-glycerol-P and L-malate oxidase activities appeared sequentially at later times and their appearance was prevented by

Table 1 Development of inner membrane oxidase activities during spore germination and outgrowth

Time of germination (min)	Oxidase activity (nmol of O_2 per min per mg protein) NADH		DL-α-Glycerol-P		L-malate	
	a	*b*	*a*	*b*	*a*	*b*
Dormant spore (0)	48	—	< 2	—	< 2	—
Heat activated spore (0)	—	85	—	< 2	—	< 2
10	—	639	—	2 6	—	< 2
20	554	676	9.8	4.3	< 2	< 2
50	728	718	39	30	2 2	2 8
100	806	1418	44	113	13	18

The sporulation medium (CCY medium) used to produce spores was a modification of that used previously[14 15] This contained $MgCl_2$ $6H_2O$ (0 5 mM), $MnCl_2$ $4H_2O$ (0 01 mM), $FeCl_3$ $6H_2O$ (0 05 mM), $ZnCl_2$ (0 05 mM), $CaCl_2$ $6H_2O$ (0 2 mM), KH_2PO_4 (30 mM), K_2HPO_4 (10 mM), glutamine (20 mg l^{-1}), acid casein hydrolysate (1 g l^{-1}), enzymatic casein hydrolysate (1 g l^{-1}), enzymatic yeast extract (0 4 g l^{-1}) and glycerol (0 6 g l^{-1}) Mature spores liberated on completion of sporulation in this medium were collected by centrifugation and repeatedly washed in deionised water (4 °C) Spore suspensions (50 mg dry weight ml^{-1}) were heat-activated in deionised H_2O at 70 °C for 30 min, allowed to cool to room temperature then resuspended in modified CCY medium plus germinants (1 mM L-alanine + 5 mM inosine) to a density of about 0 5 mg dry weight ml^{-1} The suspensions were incubated at 30 °C on an orbital shaker (220 r p m) for various times before being chilled by addition of ice cubes followed by rapid centrifugation at 4 °C The organisms were resuspended in 0 05 M Tris-HCl, *p*H 7 5 (4 °C), mixed with cooled glass beads and broken in the Braun cell disruptor for 1 min with liquid CO_2 cooling Spore integuments and occasionally a few unbroken organisms were removed by centrifuging (25,000*g* for 3 min at 4 °C) and the resulting supernatant was centrifuged at 105,000*g* for 1 h at 4 °C to deposit the membrane fraction The membrane fraction was washed once by resuspension in 0 05 M Tris-HCl (4 °C) containing 5 mM $MgCl_2$ Oxidase activities at 30 °C were determined as described previously[2] *a* and *b* refer to separate experiments

chloramphenicol Evidence is presented to suggest that in the dormant spore the electron transport sequence is blocked in the region between NADH dehydrogenase and the cytochromes

Table 1 shows that both dormant and heat-activated spore membranes had essentially the same low NADH oxidase activity. Within 5–10 min of germination there was a 5–10-fold stimulation of NADH oxidase activity (Tables 1 and 2) that probably increased further during the germination and outgrowth sequence. L-malate oxidase activity was first detectable after 50 min and had not reached full vegetative cell membrane levels of activity (approximately 50 nmol O_2 min^{-1} mg^{-1}) after 100 min DL-α-glycerol phosphate oxidase activity was detectable after 10 min and attained approximately vegetative cell membrane levels of activity (70 nmol O_2 min^{-1} mg^{-1}) during the experimental period DL-α-glycerol-P dehydrogenase activity showed a similar pattern (Table 2)

It was of interest to see whether these changes in oxidase activities were the result of protein synthesis or activation of pre-existing proteins Steinberg *et al.*[4] had reported a rapid, chloramphenicol insensitive increase in oxygen uptake in the first minutes of *B. cereus* spore germination .In an experiment where the dormant spore membranes had an NADH oxidase activity of 67 nmol O_2 min^{-1} mg^{-1} protein, the membranes of organisms germinated for 10 min in the presence or absence of chloramphenicol (100 μg ml^{-1}) had activities of 723 and 855 nmol O_2 min^{-1} mg^{-1} protein, respectively This level of chloramphenicol completely inhibits protein synthesis during germination of *B. megaterium* spores[5]. When spores were germinated with chloramphenicol for longer periods (Table 2), DL-α-glycerol P and L-malate oxidase activities did not appear, indicating a requirement for protein synthesis for incorporation of these activities into the membrane. Once again, the initial rapid increase in NADH oxidase activity was unaffected by chloramphenicol but the activity subsequently decreased after 20 min (see Table 2), indicating new protein synthesis to be necessary to maintain this level of activity later in the germination sequence

To determine whether the NADH oxidase activation occurred generally over the whole electron transport sequence, or whether a specific portion was stimulated, certain segments of the sequence were assayed using artificial electron donors and acceptors (Table 2) These experiments revealed that heat activation had no significant effect on the activity of any of the enzymes in Table 2, NADH–DCPIP reductase activity was higher than NADH oxidase activity and did not change significantly on germination, NADH–cytochrome *c* reductase activity showed a dramatic increase on germination, of the same order as the increase in NADH oxidase; and that ascorbate–TMPD oxidase activity was low and did not increase on germination. These results suggest that the electron transport sequence of the dormant spore membrane is blocked between the dehydrogenase and cytochrome *b* regions.

Room temperature reduced minus oxidised difference spectra did not reveal any rapid change in membrane cytochrome co[illegible] during germination. NADH reduced aerobic minus oxidised, liquid nitrogen temperature spectra revealed a lower steady state level of reduction of the cytochromes in the dormant spore membranes than in membranes from spores germinated for 10 min, which together with the assays of the segments of the electron transport sequence, is consistent with a block located in the sequence before the cytochromes

When spores were germinated in phosphate buffer plus germinants immediately after heat activation (Fig. 1), oxygen uptake measured in the oxygen electrode was first detectable after about 2 min The rate of uptake then increased rapidly, so that by 7 min the suspension was respiring vigorously (25 8 nmol O_2 min^{-1} mg^{-1} dry weight of spores). This oxygen uptake did not occur in the absence of germinants and was unaffected by chloramphenicol. The results were the same for spores germinated in the more complex modified CCY medium (Fig. 1). Inclusion of KF (10 mM) in the germination medium slightly delayed the onset of rapid respiration but the final rate attained was little different from that of control suspensions, as described previously[6] With KCN (10 mM) present, the maximum rate of respiration was approximately half that of the controls The rapid rate of respiration attained within the first 7 min of germination corresponds to that

Table 2 Assays of segments of the electron transport chain during germination

Time of germination (min)	NADH–oxidase			NADH–2,6-dichlorophenol-indophenol reductase			NADH–cytochrome *c* reductase			Ascorbate–TMPD oxidase			DL-α-glycerol -P oxidase			DL-α-glycerol-P-2,6-dichlorophenol-indophenol reductase		
	a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Dormant spore	99	44	—	1 68	1 79	—	69	25	—	28	43	—	< 2	< 2	—	5	9.7	
Heat-activated spore	74	—	40	1 06	—	2 38	28	—	33	26	—	41	< 2	< 2	< 2	2	6 5	15
5	377	239	—	1 9	1.44	—	175	160	—	39	39	—	< 2	< 2	—	7	4.4	
10	425	416	400	2.0	1.68	1 45	182	159	159	28	45	31	5 8	4 8	< 2	8	19.6	9
20	1048	415	202	2 3	1 28	0 79	190	135	120	53	26	26	39	16	< 2	80	45 1	5

The experimental details were essentially as in Table 1 Enzyme assays were carried out at 30 °C as described previously[2 3] Assays of ascorbate–TMPD oxidase contained 10 mM ascorbate and 0 1 mM TMPD Correction was made for the low non-enzymatic oxidation of ascorbate–TMPD *a* and *b* refer to separate experiments, in *c*, the germination was carried out in the presence of chloramphenicol (100 μg ml^{-1}) Enzyme activities are expressed in the following units NADH oxidase (nmol O_2 min^{-1} mg^{-1} protein); NADH–2,6-dichlorophenol-indophenol reductase (μmol 2,6-dichlorophenol-indophenol reduced min^{-1} mg^{-1} protein), NADH–cytochrome *c* reductase (nmol cytochrome *c* reduced min^{-1} mg^{-1} protein) Ascorbate–TMPD oxidase (nmol O_2 min^{-1} mg^{-1} protein), DL-α-glycerol-P oxidase (nmol O_2 min^{-1} mg^{-1} protein), DL-α-glycerol-P–2,6-dichlorophenol-indophenol reductase (μmol 2,6 dichlorophenol-indophenol reduced min^{-1} mg^{-1} protein)

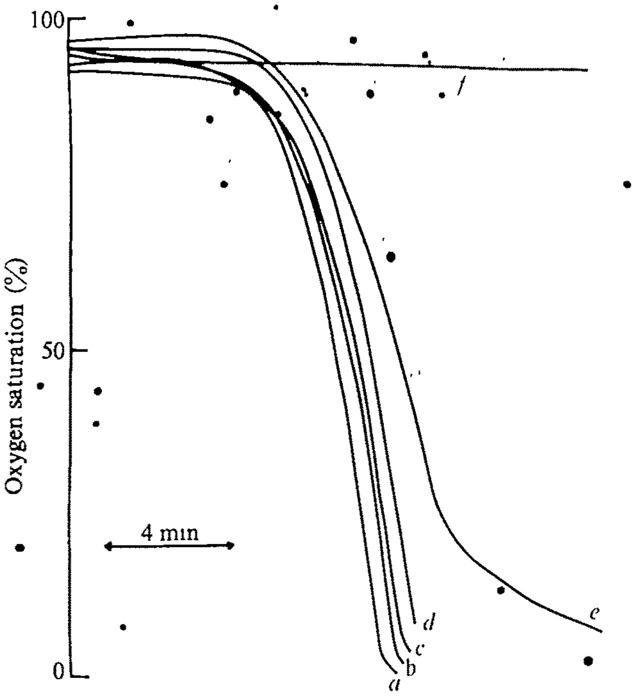

Fig 1 Aliquots of washed concentrated spores in deionised water (10 mg dry weight ml^{-1}) were heat activated by heating in a water bath at 70 °C for 30 min Immediately after heat activation, 0 2 ml of this spore suspension was added to the reaction vessel of the oxygen electrode Incubations were as follows 50 mM KH_2PO_4/K_2HPO_4 (pH 7.5) containing 1 mM L-alanine and 5 mM inosine (*b*), *c*, *d* and *e* repeats of (*b*) with addition of 100 μg ml^{-1} chloramphenicol, 10 mM potassium fluoride and 10 mM potassium cyanide, respectively, *f*, result of incubation in 50 mM KH_2PO_4/K_2HPO_4 (pH 7 5) alone. The final incubation volume was 3 ml in all cases and the reaction vessel temperature was maintained at 30 °C

observed by Steinberg *et al*[4] and correlates with the observed increase in membrane NADH oxidase activity.

This rapid chloramphenicol-insensitive activation of the respiratory membrane of the spore on germination seems to be a general property of *Bacillus* spores, since similar results to those described here have been obtained for all species so far examined including *B. cereus* and *B subtilis* The activation also occurs in the absence of membrane lipid synthesis which is not detectable until 10 min after germination[7]. When spores were germinated in 40 mM Tris-HCl buffer containing either 1 mM L-alanine or 40 mM calcium-dipicolinic acid[8], the same pattern of NADH oxidase activation was observed. Since, in these conditions the spores could germinate but not outgrow, it would seem that the activation is not inextricably linked to spore outgrowth.

The biochemical basis of the suggested block in the dormant spore electron transport chain now remains to be determined Possible mechanisms to account for its rapid removal during germination include degradation or dissociation of an inhibitor and membrane conformational changes resulting from the expansion of spore membrane which is thought to occur early in germination. Although lipid synthesis is not detectable until 10 min after germination is initiated, turnover of existing spore membrane lipids starts almost immediately on initiation and is therefore occurring the period of NADH oxidase activation[7].

Rapid increases in respiratory rates upon termination of the cryptobiotic stage have also been recorded for fungal spores[9], and insects in diapause[10]. To what extent activation of pre-existing enzymes is involved in these instances is not known. Activation dose seem to have a role in sea urchin eggs after fertilisation, where the sudden increase in respiratory activity was found to be insensitive to chloramphenicol and actinomycin D (refs 11 and 12). In etiolated primary leaves of *Phaseolus vulgaris*, green chloroplasts formed during exposure to short light flashes did not exhibit Photosystem II activity. These chloroplasts, however, acquired the full Photosystem II activity within minutes after exposure to continuous light, suggesting that some form of activation is occurring[13] It is not known whether this activation is independent of protein synthesis

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Solubilisation of atropine-binding material from brain

MUSCARINIC receptors have been characterised extensively in intact smooth muscle[1–3] and in subcellular fractions from smooth muscle[2,4–6] and brain[7–10] by reversible binding of labelled atropine[1,8,10], propylbenzilylcholine[9,10], quinuclidinylbenzilate (QNB) (refs 6, 8) and benzetimide[4,5], or by alkylation with benzilylcholine[2] or propylbenzilylcholine mustard[3,7,9–11]. Several attempts to solubilise muscarinic receptors[2,4,12] have failed due to the inhibition of receptor binding by the detergent used for solubilisation[2,4]. We report here the solubilisation with 2M sodium chloride of an atropine-binding material from the ox cerebral cortex, which has properties similar to those found for the muscarinic receptor in subcellular preparations.

Figure 1*a* shows the binding of (±) ^{3}H-atropine to a crude synaptosomal preparation isolated from the cerebral cortex; the measured dissociation constant (K_D) was 2.0±0.3 nM, the Hill coefficient n_H was 1.0 and the concentration of the binding sites was 2.0±0.5 nmol g^{-1} protein. The high affinity binding of labelled atropine could be displaced by unlabelled atropine, scopolamine, lachesine and QNB. The estimates of the inhibition constants (K_i) of these antagonists, together with those of agonists (determined in this study and by other groups) are shown in Table 1 There was close agreement between the constants measured in our preparations and those reported by others although, in our hands, QNB showed two distinct affinities ($K_i = 2\times10^{-10}$ M and 3×10^{-8} M) of which only the higher affinity value has been reported before[8], possibly because its binding had not been studied at sufficiently high concentrations.

Figure 2 illustrates the inhibition by acetylcholine of binding of labelled atropine to the membrane preparations. The inhibition curve indicates two binding sites for acetylcholine: a high affinity site with $ID_{50} = 10^{-5}$ M and a lower affinity site with $ID_{50} = 10^{-3}$ M. These values correspond to values of K_i of 3×10^{-6} M and 3×10^{-4} M, respectively. Three other agonists, acetyl-β-methylcholine, oxotremorine and carbamylcholine gave similar biphasic curves for inhibition of atropine binding. Their affinity constants derived from the competition with ^{3}H-atropine were respectively 3×10^{-6} M and 10^{-4} M, 3×10^{-7} M and 2×10^{-5} M, and 2×10^{-6} M and 2×10^{-4} M We found that the high-affinity binding component represented 35–45% of the total number of atropine-binding sites, a figure similar to the 32% reported by Hulme *et al.*[9,10]. These two components of the curve could represent identical muscarinic binding sites under different membrane restraints, or alternatively, only the high affinity site may be related to the receptor. A single binding constant was found for pilocarpine and arecoline (10^{-6} M and 2×10^{-6} M, respectively) and choline was ineffective up to millimolar concentrations. The K_i values found for both agonists and antagonists agree reasonably well with those reported by Yamamura *et al.*[8] for subcellular brain preparations and with the agonist binding associated with smooth muscle contraction[1,13]. Drugs unrelated to the muscarinic system generally did not displace the binding of labelled atropine.

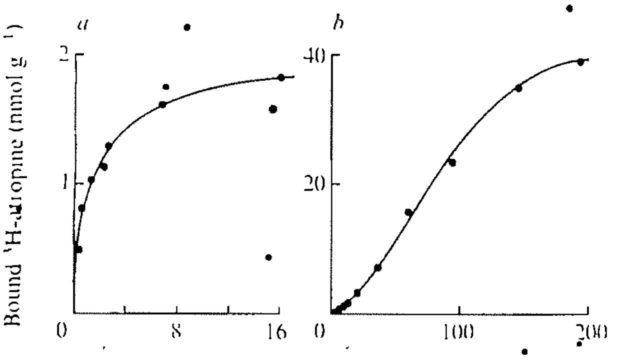

Fig. 1*a*, Binding of ^{3}H-atropine to a crude synaptosomal preparation from ox cerebral cortex The subcellular fraction was the P_2 pellet of Gray and Whittaker[14]. A sample (0.25 ml) of the P_2 suspension in 30 mM sodium phosphate buffer containing 100 mM sodium chloride, *p*H 7.4, at a protein concentration of 0.5–1.5 mg ml^{-1} was incubated at the room temperature for 10 min with ^{3}H-atropine with and without unlabelled atropine. A 200-µl sample was withdrawn and filtered on Millipore membranes and washed with 20 ml cold distilled water. Because the 'off' reaction of atropine from receptor is slow, the quantity of drug removed by this wash from the receptor should be very small. The filter was dried, then counted by liquid scintillation spectrometry in toluene–POPOP. The specific binding shown in the graph represents the difference in counts between uninhibited and inhibited (1 µM atropine) samples. $K_d = 2.0$ nM; $n_H = 1.0$. Apart from the binding site shown, two others were detected at higher concentrations of atropine, with $K_d = 4\times10^{-7}$ M and $K_d = 6\times10^{-6}$ M, their capacities being 20 and 180 nmol ^{3}H-atropine bound per g of protein respectively These two sites were not susceptible to muscarinic agents and thus unlikely to be related to the muscarinic receptor. (±) ^{3}H-atropine (170 mCi mmol^{-1}) was purchased from the Radio Chemical Centre, Amersham. *b*, Binding of ^{3}H-atropine to the solubilised receptor. The solubilised material was prepared as follows. Equal volumes of P_2 suspension (30 ml, at a protein concentration of about 10 mg ml^{-1}) and 4 M NaCl were mixed—giving a final NaCl concentration of 2 M—and the suspension was stirred vigorously for 2–3 h at 4 °C. It was then centrifuged at 15,000 r p.m. (28,000*g*) for 20 min, and the supernatant was diluted fourfold with 100 mM phosphate buffer *p*H 7.4 and recentrifuged at 25,000 r.p.m. (83,000*g*) for 2 h in the SW 27 rotor of a Beckman L5–40 ultracentrifuge To the resulting supernatant was added ammonium sulphate (55 g per 100 ml, over a period of 1 h during which the medium was stirred constantly) and the material was then centrifuged at 15,000 r.p.m. for 20 min to obtain the precipitated proteins. The combined pellets were dissolved in 5 ml 100 mM phosphate buffer *p*H 7.4, and dialysed exhaustively to remove ammonium sulphate. Finally, the dialysate was centrifuged at 15,000 r p.m. for 30 min and the supernatant (referred to as the solubilised material) was assayed for ^{3}H-atropine binding. About 5% of the total membrane proteins was solubilised. Proof that the material was soluble came from observations that no particulate matter was visible under the electron microscope and that the material did not sediment when centrifuged at 83,000*g* for 2 h. Binding of ^{3}H-atropine to the solubilised material was measured by rapid filtration on cellulose nitrate membranes (Selectron-Filter, type BA 85, 0.45 µm, Schleicher and Schull). Free ^{3}H-atropine was removed by washing the membranes with 20 ml cold distilled water. We could detect no binding if the solubilised material was heated for 10 min at 60 °C before binding assay. $K_{1/2} = 80$ nM; $n_H = 1.5$. From comparisons of atropine binding to the soluble material and to the P_2 fraction, before and after salt-treatment, we found that the number of solubilised binding sites was equal to 50±10% of the high-affinity P_2 sites (assuming quantitative absorption on to the cellulose nitrate membranes). Binding of ^{3}H-atropine to the solubilised material is being studied by equilibrium dialysis to determine the effectiveness of the cellulose nitrate membranes in retaining the soluble material ^{3}H-atropine binding to the solubilised material was unaltered after storage at 4 °C for 5 d.

After treatment of a crude synaptosomal preparation with 2M NaCl and separation of the solubilised material by centrifugation, binding of ^{3}H-atropine could be detected in the solubilised fraction by a filtration assay (Fig 1*b*). The binding curve was sigmoidal ($n_H = 1.5$), half-saturation ($K_{1/2}$) occurring at 80±30 nM, and the concentration of binding sites was 40±20 nmol g^{-1} protein.

The concentrations of unlabelled antagonists which reduce by 50% the level of specifically-bound ^{3}H-atropine (80 nM) (ID_{50} values) were found to be between 20 and 50 times higher than the corresponding concentrations found in synaptosomal preparations (Table 1). A single class of binding sites was found for each antagonist tested, the Hill coefficient being slightly greater than one (1.3–1.5) Competition by agonists of ^{3}H-atropine-binding was studied in the same way, as shown in Fig. 2 for acetylcholine. A single binding component was observed with $ID_{50} = 10^{-6}$ M: the Hill coefficient was 1.2. This competition was similar to the high affinity component observed for membrane preparations (Fig. 2). Oxotremorine, acetyl-β-methylcholine and carbamylcholine also displayed single binding constants (ID_{50} of 10^{-6} M, 10^{-5} M and 6×10^{-6} M, respectively).

The solubilised material thus contained a single, high affinity binding site for agonists with an ID_{50} value which closely parallelled that of the high-affinity component in the subcellular preparation. The low-affinity site was either lost, inactivated, left unsolubilised or converted to the higher affinity form. Antagonists bound to the soluble fraction with QNB being the most active, followed by scopolamine, atropine and lachesine—the same order as binding to the membrane fraction—but in a sigmoidal fashion and with lower affinities than to the membrane preparations. Such cooperativity may represent a transition of the soluble protein from a state of low to high affinity towards

Fig. 2 Inhibition of specific ^{3}H-atropine-binding to the P_2 fraction and to solubilised material by acetylcholine in the presence of 1 µM eserine sulphate The concentration of the ^{3}H-atropine was 4 nM for the P_2 fraction and 80 nM for the soluble fraction. Binding was measured as described in the legend to Fig. 1. ○, P_2 fraction, ●, P_2 fraction—high-affinity binding component; ×, solubilised material.

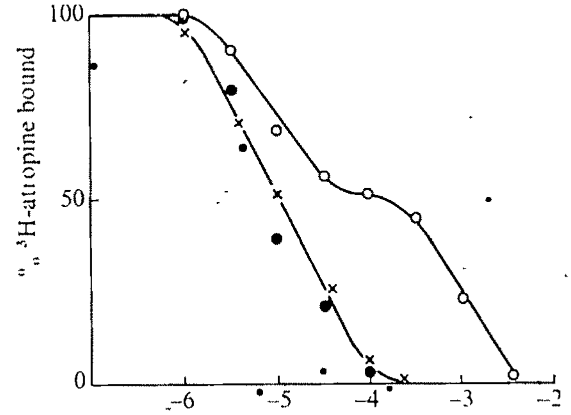

Table 1 Binding characteristics for membrane-bound and soluble atropine-binding material

Drugs	Particulate preparations Rat brain* ID_{50} (M)	Rat brain† ID_{50} or K_i (M)	Ox brain§ K_i (M)	Soluble material Ox brain§ ID_{50} (M)
(±) Atropine	$10^{-9}-2\times10^{-9}$ (8)	1.2×10^{-9} (11)	2×10^{-9}	8×10^{-8}
(±) Scopolamine	$8-9\times10^{-10}$ (8)	6×10^{-10}‡	9×10^{-10}	6×10^{-8}
Lachesine	—	1.5×10^{-9} (11)	3×10^{-9}	15×10^{-7}
(±) QNB	$4-5\times10^{-10}$ (8)	2×10^{-10} (11)	2×10^{-10}	4×10^{-9}
Acetylcholine	$2-4\times10^{-6}$ (8)	3.3×10^{-7} (9, 10) 2.5×10^{-5} (9, 10)	3×10^{-8} 3×10^{-6} 3×10^{-4}	10^{-5}
(±) Acetyl-β-methyl choline	$3-5\times10^{-6}$ (8)	8×10^{-7}‡ 6.6×10^{-5}	3×10^{-6} 10^{-4}	10^{-5}
Oxotremorine	$5-8\times10^{-7}$ (8)	4.6×10^{-7}‡	3×10^{-7} 2×10^{-5}	10^{-6}
Carbamylcholine	$2-3\times10^{-6}$ (8)	5×10^{-7} (9, 10) 1.5×10^{-5} (11)	2×10^{-6} 2×10^{-4}	6×10^{-6}
Arecoline	$1-3\times10^{-6}$ (8)	10^{-7}‡ 10^{-5}‡	$2\times10^{-}$	4×10^{-5}
Pilocarpine	7×10^{-6} (8)	6.7×10^{-6}‡	10^{-5}	2×10^{-6}

From competition curves with ^{3}H-atropine, apparent inhibition constants were obtained for the membrane-bound material, according to the equation $K_i = ID_{50}/(1+C/K_d)$ where C is the concentration of radioactive ligand and K_d its dissociation constant (the concentration of (±) ^{3}H-atropine was 4–6 nM). In the case of the soluble preparation, ID_{50}-values are reported because of the complex nature of the atropine-binding curve (Fig. 2). (±) ^{3}H-atropine concentration was 80 nM The following compounds had no effect on binding of labelled atropine at 20 µM: choline, *d*-tubocurarine, gallamine, α-toxin from *Naja naja nigricollis*, decamethonium, eserine, neostigmine, edrophonium and Tetram ((2-diethylamino)ethylphosphorothioic acid, 0,0-diethyl ester).

*All values are ID_{50}, that is the concentration of drugs which reduced ^{3}H-QNB binding by 50% (concentration of ^{3}H-QNB was 6×10^{-11} M). Reference numbers are given in parentheses

†Values given are either dissociation constants (obtained from the reciprocal of the quoted affinity constant (ref. 11 and personal communication from N. J M. Birdsall)) or ID_{50} values [9,10], all derived from ^{3}H-propylbenzilylcholine displacement.

‡Personal communication from N. J. M. Birdsall.

§Results from the study reported here.

antagonists. Analogous changes in binding properties have been observed in other systems after solubilisation, notably in the case of the nicotinic receptor[15]. These results suggest that the solubilised material which shows specific atropine binding was derived from the high-affinity component of the membrane preparation which is most likely to be the muscarinic receptor site.

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Calcium levels in the brain underlie temperature control during exercise in the primate

ALTHOUGH it is known that the hyperthermia in man accompanying steady-state submaximal exercise is a regulated rise which is virtually independent of the ambient temperature[1], a fundamental question persists. What are the neural mechanisms involved in this regulated rise and in the ensuing hyperthermia encountered during severe work in the heat? It has been proposed that the elevation in the temperature set-point associated with fever has as its basic component a change in the hypothalamic balance between the essential cations, Na and Ca^2 (refs 2, 3) Is it possible that such a mechanism could underlie the shift in temperature set-point which occurs during exercise? To explore this idea, we undertook two types of experiments. First, an artificial cerebrospinal fluid (CSF) containing excess Ca^{2+} ions was infused into the third ventricle. Secondly the kinetics of endogenous Ca^2, as reflected by its retention or release into the third ventricle, was analysed We report here that Ca^{2+} ions, acting directly on the brain, can indeed attenuate or entirely block the hyperthermia of exercise without necessarily interfering with work output; further, the actual efflux of Ca^{2+} ions from the diencephalon increases markedly during the exercise-induced hyperthermia

Macaque monkeys were used, as the heat-regulating processes in man and subhuman primates are similar[4]. Two monkeys were seated in primate chairs and trained to exercise strenuously on a 'rowing machine'[5], using food pellets as reinforcers, so that they lifted up to three times their own body weight. Once the work rate had stabilised for an interval of 30 min, over several days, surgery was undertaken Following neurosurgical procedures[6] an array of three cannulae was implanted aseptically with the tips resting in the third ventricle, their patency was verified by the standard CSF pulsatile method On recovery, the monkeys were exercised again for two 30-min periods every day for 2 weeks before infusions were undertaken

When 0.5 ml of CSF was infused into the third ventricle at a constant rate over 10 min, the monkey continued to exercise, and its typical exercise-induced rise in colonic temperature persisted unabated. As shown in Table 1, however, the addition of excess Ca^{2+} ions to the infusion medium attenuated the hyperthermia in a dose-dependent manner In one monkey (*A*), the elevation in colonic temperature was reduced by 72%, yet total work output was reduced by only 37%. In another monkey (*B*), the rise in its body temperature due to exercise was virtually blocked (+0.2 °C) after the infusion of excess Ca^{2+} ions, yet the decline in total work performed was less than 25%. In both cases, when Ca^{2+} was infused into the monkey's ventricle in a sufficient concentration to induce a hypothermia, the monkey nevertheless exercised to a substantial degree On the basis of X radiographs taken in the sagittal plane, the differences in location of the tips of the cannulae may account for the differences in the animal's responses.

Table 1 Changes in colonic temperature from baseline control and total amount of work performed during exercise in neutral, cold and hot environments

Neutral (22 °C db)	Solution	Temperature change (C)	Total work (J per 30 min)
Monkey A	Control 5-ion	+1.45	2018
	Ca^{2+} 2.5 mM	+0.40	1258
	Ca^{2+} 10 mM	−0.45	1018
Monkey B	Control 5-ion	+1.35	2795
	Ca^{2+} 10 mM	+1.05	2556
	Ca^{2+} 15 mM	+0.20	2181
	Ca^{2+} 20 mM	−0.40	1045
Heat (35 °C db) Monkey A	Control 5-ion	+1.10	1338
	Ca^{2+} 2.5 mM	+0.45	1192
Cold (0 °C db) Monkey A	Control 5-ion	+0.55	2036
	Ca^{2+} 2.5 mM	+0.05	1367

To examine the effect of ambient temperature, we changed the environmental temperature of the monkey by a stream of hot or cold air blown into its chair 15 min before exercise and for the 30 min interval of exercise. In one monkey (*A*) we raised the ambient temperature to 35 °C and found that the excess calcium infusion (Table 1) greatly attenuated the exercise-induced rise in temperature without markedly affecting the amount of work performed. On the other hand, after the same monkey's ambient temperature was lowered to 0 °C (Table 1) on another day, its usual rise in temperature was abolished, but its work output was reduced by only 33%.

To determine whether a physiologically-induced change in endogenous calcium actually occurs within the diencephalon of the monkey as it exercises, we radiolabelled the structures surrounding the third ventricle by injecting 6.0–8.0 μCi of $^{45}Ca^{2+}$, in a volume of 6 μl, directly into the third ventricle on the day before an exercise experiment began. Before the 30 min of intense exercise, a series of 5-min push–pull perfusions of the tagged region were carried out at 15-min intervals in order that a stable washout curve of $^{45}Ca^{2}$ radioactivity could be generated. As soon as each monkey began to exercise, the efflux of $^{45}Ca^{2+}$ into the perfusate rose sharply from 100 to 300% (Table 2). At the same time, the typical exercise-induced rise of temperature occurred. Following the cessation of work, the augmented level of $^{45}Ca^{2+}$ in the perfusates (Table 2) continued for the next two to five perfusions, which corresponded to the interval of elevated body temperature.

Table 2 Percentage increases in $^{45}Ca^{2+}$ activity above the baseline d.p.m. in perfusate collected during 30 min exercise ($N = 2$) and in the 1 h post-exercise interval ($N = 3$)

	During exercise	After exercise
Monkey A	163%	131%
Monkey B	249%	283%

Our experiments provide new evidence that Ca^{2+} is involved in the shift in temperature which occurs during exercise. In both the rat and dog exercising on a treadmill, excess Ca^{2+} ions infused intracranially also attenuate the usual rise in the animal's temperature[8,9]. Therefore, it seems possible that the set-point for body temperature during exercise could be mediated by ions within the diencephalon[2], and that the set-point shifts upwards mainly by means of an unbinding, change in transport, or other activity of Ca^{2+}.

It is clear that the rise in central body temperature during exercise[9] can be modified or entirely abolished without necessarily incapacitating the animal or preventing it from performing a given work activity. These findings may have important implications for the clinical problems associated not only with the mechanism underlying heat stroke, but also with runaway hyperpyrexia.

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Chorionic membrane as an extra-adrenal source of foetal cortisol in human amniotic fluid

STUDIES in animals suggest that foetal cortisol (F) may have an important role in foetal lung maturation and the mechanism of parturition. In man the situation is complicated by the greater permeability of the placenta to this hormone[1] and its intraplacental conversion to its biologically inactive metabolite, cortisone (E) (refs 2–4). Many other foetal tissues also convert F to E (ref. 5). As a result, human foetal blood contains a relatively large amount of E, and a lower F : E ratio. Recently, we observed that the F : E ratio of amniotic fluid is considerably higher than that of cord serum[6]. To explore this, the possible influences of the foetal kidney, skin, gastro-intestinal tract, lung and membranes were investigated; only the chorionic membrane was found to convert cortisone to cortisol.

Foetal tissues and fluids were obtained at hysterotomy in patients undergoing abortions for maternal indications from 11–22 weeks gestation, at elective Caesarean section and vaginal delivery at term and from two anencephalic stillbirths. Placenta and membranes were also obtained at vaginal delivery and amniotic fluids at amniocentesis. Endogenous F and E concentrations in fluids and homogenised tissues were assayed after Sephadex LH-20 column chromatography, by radiotransinassay using dog transcortin as described previously[7,8]. For the enzyme studies, pieces of tissue (0.5–1 g) were weighed, minced (except for amnion and chorion which were left intact) and added to 2.0 ml medium (0.9% saline, amniotic fluid, Krebs–Ringer bicarbonate glucose buffer, or phosphate buffer, *p*H 7.4). Since similar results were obtained for all media, later studies used saline only. Incubations were carried out for 2 h at 37 °C in glass counting vials containing tracer amounts (100 pg) tritiated F or E with or without added cold steroid. Following addition of 10 ml ethyl acetate the vials were capped and shaken vigorously for 10 min. Organic phase 5 ml was evaporated to dryness under air, dissolved in 0.2 ml methylene chloride: methanol 98 . 2 and applied to Sephadex LH-20 columns[8]. This procedure separated the F and E fractions so that the radioactivity in each could be determined. Conversion was calculated as 100 × c.p.m. $_{product}$/c.p.m. $_{product + substrate}$, less the control value calculated in the same way. Recovery of radioactivity as E+F was 80–100% that of the controls which consisted of tracer in medium prepared and incubated along with the tissues. Alternative controls consisted of identical tissue preparations placed in a boiling water bath for 5 min before

Table 1 F and E in foetal fluids (ng ml^{-1})

Gestational age (weeks)		Amniotic fluid				Cord arterial serum				Cord venous serum			
		n	F	E	F/E	*n*	F	E	F/E	*n*	F	E	F/E
11–16	Mean	11	3.0	9.2	0.33*	2	12.2	32.5	0.47	2	6.5	52.3	0.18
	s.e.m.		0.6	1.6	0.04								
17–22	Mean	11	4.7	6.7	0.66*†‡	6	6.7	17.3	0.35†	6	3.7	20.7	0.15‡
	s.e.m.		0.9	1.0	0.07		2.6	4.5	0.11		1.9	7.6	0.02
25–30	Mean	4	9.4	5.1	1.8								
	s.e.m.		1.7	1.1	0.70								
35 (anencephalic)	No. 1	2	24.7	28.0	0.88	2	15.6	80.0	0.20	2	39.7	163	0.24
	No. 2		16.3	16.0	1.02		8.9	60.5	0.20		13.7	73	0.19
36–40 (no labour)	Mean	7	18.0	10.6	3.8	5	36.0	62.0	0.57	5	20.5	70.5	0.29
	s.e.m.		3.0	2.6	1.8		7.1	6.9	0.11		5.6	3.6	0.08
36–40 weeks (Spontaneous labour)	Mean	5	28.5	18.9	1.5	6	120	108	1.2	6	98	143	0.76
	s.e.m.		8.3	7.1	1.0		25	15	0.4		27	17	0.11

*$P<0.001$; †$P<0.05$; ‡$P<0.001$.

incubation. Identity of the products isolated from membranes and placenta was confirmed by recrystallisation to constant specific activity.

To simulate conditions *in vivo* more closely, four experiments were done in which layers of chorion and amnion from term placentas were placed over small shallow chambers 5 cm in diameter, tracer in 10 ml amniotic fluid was placed in the cavity lined by amnion, and the dishes were incubated for 2 h at 37 °C. Qualitatively similar though quantitatively about half the conversion seen in the routine incubation conditions was observed. When 10 ml saline was placed on the chorionic side, about one-third of the total activity passed through, mainly in the converted form. When the tracer was placed in the saline, about one-third passed into the amniotic fluid, again mainly as cortisol. These studies show that steroids can pass through the membranes from either the maternal of foetal side. It seems likely, however, that diffusion between uterus and chorion would be slow, whereas that between amnion (and chorion) and amniotic fluid would be hastened by the movement of the amniotic fluid itself.

Table 2 Interconversion of F and E in foetal tissues (%)

Tissue	Gestational age (weeks)	F → E	E → F
Kidney	12	39	3
	20	55	0
	35 (An)	83	3
Skin	12	24	4
	20	59	3
	35 (An)	17	2
Cord	20	3	2
GI tract			
All	12	20	3
Small bowel	20	27	17
Stomach	35 (An)	12	1
Small bowel	35 (An)	20	0
Large bowel	35 (An)	1	2
Lung	12	54	3
	18	56	10
	35 (An)	22	3

An, Anencephalic.

Table 1 compares the endogenous concentrations of steroids found in cord serum and amniotic fluid. The F : E ratio was consistently higher in amniotic fluid than in cord serum at all gestational ages and rose with increasing age. F levels equalled or exceeded E levels in amniotic fluid from 25 weeks onward.

Table 2 shows the interconversion of F and E in several foetal tissues at various gestational ages. Conversion of F to E usually occurred while conversion of E to F was negligible, results in accord with previous studies[5] and with the endogenous concentrations shown in Table 3. The latter showed lower F:E ratios than serum at all ages studies.

In Fig. 1, the interconversions of F and E in chorion and in placental cotyledons are compared. The placenta showed high conversion of F to E throughout pregnancy. The reverse conversion occurred in the chorion with high values from 16.5 weeks until after labour had started, when they fell. The back reactions were consistently low averaging 65% for placental E→F and 10.2% for chorionic F→E.

Table 3 Endogenous F and E in foetal tissues (ng g^{-1})

Tissue	Gestational age (weeks)	F	E	F:E
Kidney	12	2.2	24.0	0.09
	20	0.3	13.4	0.02
	35 (An)	4.1	61.4	0.02
Skin	35 (An)	0.3	10.7	0.03
GI tract				
Stomach	35 (An)	0.5	14.0	0.04
Small bowel	35 (An)	0.7	21.3	0.03
Large bowel	35 (An)	0.5	10.0	0.05
Lung	12	1.3	21.7	0.06
	20	1.3	16.8	0.08
	35 (An)	2.7	18.8	0.14

An, Anencephalic.

Conversion in both tissues was greatly decreased by boiling. No differences were seen in conversion in either direction when 100 ng ml^{-1} F or E was added to the incubation medium of three placentas and three chorionic membranes (two from early pregnancy, one from late pregnancy). Little or no activity was seen in the amnion. When the chorionic membranes were examined microscopically, a layer of

Fig. 1 F–E interconversion: in placenta F→E (○) and chorion E→F (●). An, Anencephalic; dotted lines, mean trends.

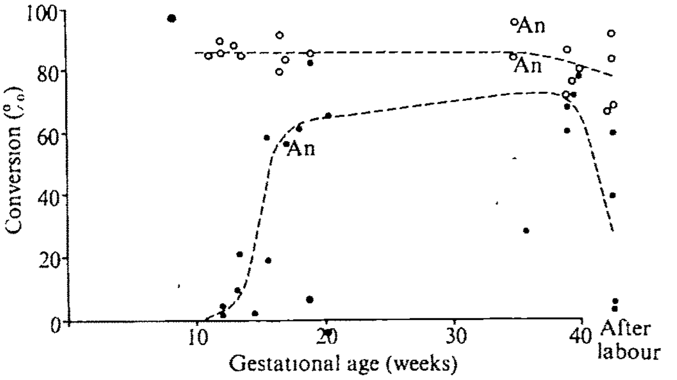

Table 4 Endogenous F and E concentrations in placenta and chorion (ng g^{-1})

		Placenta			Chorion		
Gestational age	(Weeks)	F	E	F : E	F	E	F : E
Early pregnancy	13.5	2.0	183	0.012	13.7	25.0	0.55
	16.5	0.8	50	0 015	4 6	21.5	0.21
	16.5	3.0	117	0.026	69 5	2.0	34.7
	17	3 0	103	0.029	21.6	4.0	5.4
	19	2.5	60	0.042	28.0	0.6	44.8
Premature (anencephalic)	35	12.9	295	0.044	194	47.0	4.1
	35	5.4	160	0 034	54	19.2	2.8
Term	38+	—	—	—	11.4	0.6	18.0
	39	21 9	57	0.38	59 0	14.0	4.2
	40	7 5	489	0.015	16.3	4.0	4.1
	40	7 9	226	0.034	52.5	16.1	3.3
	40	6.7	253	0.027	43.5	11.5	3.8
Mean				0 06			12.5*
s.e m.				±0.03			±4.8

*Excluding values from 12–16 5 weeks with F : E < 1.

maternal decidua was frequently adherent. Membranes from which this material was carefully scraped off retained their original activity The uterine wall was also found to contain the same or a similar enzyme and this is currently under investigation[9] No significant conversion of E or F was found when chorionic membrane from a single guinea pig killed under ether anaesthesia at 58 d gestation was incubated similarly.

The results of these *in vitro* studies are further substantiated by the endogenous levels shown in Table 4. A high F E ratio was seen in chorion and a much lower ($P<0.02$) one in placenta

These observations show that the chorionic membrane is a metabolically active tissue which behaves differently from placental cotyledons. The rise in chorionic conversion of E to F in early pregnancy corresponds to the rise at 15–20 weeks in amniotic fluid cortisol which we reported previously[11]. This chorionic conversion of E to F accounts also for the rising F : E ratio in amniotic fluid relative to cord serum observed through pregnancy The declining activity in labour accords with the lesser change in amniotic fluid F E ratio during labour despite the surge of F in cord blood associated with its spontaneous onset.

These results may explain the poor correlation we have found between amniotic fluid cortisol and lecithin or the palmitic : stearic ratio (ref. 11). Amniotic fluid cortisol is mostly diffusible[12] and is presumably taken into the foetus by swallowing or other means, possibly by diffusion into the lung. In sheep the lung is known to produce fluid steadily, making absorption of cortisol unlikely[13] In man there are few data and these are controversial[14]. Since the chorion in anencephalic infants seems to be normally active and since some of these infants breathe at birth, absorption of amniotic F through the lung or open cranium, might account for the relatively normal lung development seen in some anencephalics despite poor adrenal function[15].

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Spectrin is absent in various tissue culture cells

A HIGHLY sensitive radioimmunoassay has been developed for human spectrin, one of the major components of the erythrocyte membrane[1–6]. We report here use of this assay on cells of human, monkey and rat origin and show that spectrin is absent from all cell lines studied, with the exception of erythrocytes. These findings seem important when models which invoke spectrin to regulate membrane fluidity,[7,8] or membrane-cytoskeletal interaction[9,10] are considered.

Spectrin is extracted from erythrocyte ghosts by alkaline solutions of low ionic strength[3] Purified spectrin consists of two high molecular weight polypeptides (molecular weights of 250,000 and 220,000) that are different from the myosin heavy chains[2,3] The properties that make spectrin of major interest for generalisation about a specific membrane protein are its abundance in the membrane protein fraction (approximately 25% of membrane protein)[1–5] and its localisation exclusively on the cytoplasmic side of the erythrocyte membrane[5,6] In addition, it is generally assumed that spectrin has an important role in regulating the mobility of transmembrane particles in the plane of the membrane[7,8] and that an interaction between spectrin and actin is involved in maintaining the shape and morphology of the erythrocyte[9,10] Particularly interesting in this respect are reports on the association of spectrin and actin and the effects of spectrin on the *in vitro* polymerisation of actin[11,12] Proteins with 'spectrin-like' properties (that is, high polypeptide molecular weight, solubility in low salt, and lack of myosin-like ATPase activity) have been found in echinoderm sperm[13], sea urchin eggs[14], macrophages[15] and *Acanthamoeba*[16] where they are also assumed to regulate the polymerisation of actin. These studies suggest that spectrin may be an ubiquitous protein in eukaryotic cells. Painter *et al* [10], however, were unable to detect spectrin by complement fixation in HeLa cells or in human fibroblasts, lymphocytes or platelets at a level higher than the background of the assay which was approximately 0.1% of the total cell protein. This assay was not very sensitive because even in erythrocytes spectrin accounts for only approximately 0.3% of the total cell protein[5] and because a 'spectrin-like' actin-binding protein has been found at the 1% level[15] Concentrations of spectrin corresponding to 5% of the myosin content of most non-muscle cells would not have been detected, so the data of Painter *et al* [10] do not exclude a potential

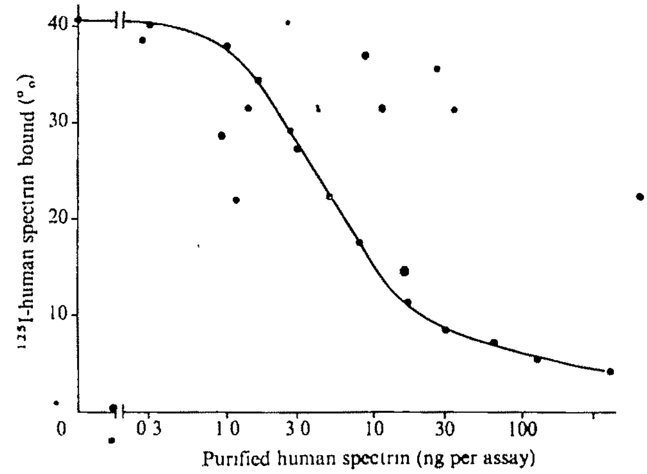

Fig. 1 Competition of unlabelled with ^{125}I-labelled human spectrin for binding to a specific antibody. Purified spectrin was iodinated in a reaction mixture (60 μl) to which 2 μg human spectrin, 0.5 mCi carrier-free Na ^{125}I, 25 μg Chloramine T, and 62 μg $Na_2S_2O_5$ were sequentially added[18]. After separation of the labelled protein and unreacted Na ^{125}I by gel filtration on Sephadex G-25, the radioactively labelled spectrin was rechromatographed on Sepharose 4B in order to eliminate some aggregated material. The specific activities of the preparations varied between 45 and 100 μCi per μg of spectrin Unlabelled human spectrin (0.2 ml) at various concentrations was preincubated (18 h at 4 °C) with 0.1 ml of a crude IgG fraction (21.4 mg protein per ml; diluted 8000-fold) of rabbit anti-human spectrin After addition of 0.1 ml ^{125}I-spectrin, equivalent to about 0.2 ng of protein, incubation was continued for 48 h Carrier rabbit IgG and enough sheep anti-rabbit IgGs were added to precipitate the formed antigen–antibody complexes maximally (24 h at 4 °C) Labelled spectrin and the various IgG-dilutions were kept in buffer containing 50 mM Na-phosphate, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1 mM phenylmethane sulphonyl fluoride (PMSF), 0.02% Na N_3 and 0.2% bovine serum albumin (BSA). Unlabelled spectrin was diluted with 30 mM Tris-HCl, pH 8.0, 1% Triton X-100, 0.1 mM EDTA, 0.1 mM PMSF and 0.2% BSA The protein concentration of pure spectrin was determined using the extinction coefficient ε280 nm = 1.01 ml cm^{-1} in mg^{-1} (ref 2)

function of spectrin as a regulatory protein of the microfilament system.

In order to increase the sensitivity of a spectrin-specific assay we have developed an immunological competition assay in which unlabelled spectrin competes with highly radioactively labelled spectrin for binding to a limited amount of spectrin-specific antibody (Fig. 1). Spectrin was purified from human erythrocyte ghosts by standard procedures[17]. Gel filtration on Sepharose 4B removed not only impurities but also erythrocyte actin. The resulting preparation showed only the two high molecular weight polypeptides even on heavily overloaded polyacrylamide gels containing sodium dodecysulphate. Antibodies against native spectrin were raised in rabbits The antisera formed a single precipitin band on immunodiffusion against human spectrin.

The radioimmunoassay (Fig. 1), which to our knowledge is the first one for spectrin, can detect as little as 5 ng of erythrocyte spectrin per ml. Thus in extracts of human cells this assay should detect spectrin at levels as low as 10^{-4}–10^{-3}% of total protein.

Table 1 summarises our results on the radioimmunological detection of spectrin in extracts of various tissue culture cells of human and monkey origin Spectrin could not be detected antigenically in any of the cell lines studied, including both fibroblasts and epithelial cells. HeLa cells, grown either in suspension or in monolayer were devoid of spectrin, in spite of the differences in morphology and cell shape present in the different growth conditions. In these cells the assay would detect spectrin-cross-reacting material if there were more than approximately 250 molecules of spectrin in a single cell. Spectrin added exogenously to the cell homogenates was easily detected in the radioimmunoassay and the estimated values were in good agreement with the known input (see Table 1). Thus potential loss of spectrin due to proteolytic degradation during the long incubation time used can be ruled out. For intact erythrocytes Bretscher[5] reported a ratio of 45 mg haemoglobin to 0.6 mg membrane proteins; assuming about 25% of the mass of the membrane proteins to be spectrin leads to a value < 0.32% of the total erythrocyte protein being spectrin Our estimation (0.24%) is in good agreement with this calculation, suggesting that in the conditions used spectrin is almost completely extracted from the cells

In contrast to an earlier antibody preparation[20], our rabbit anti-human spectrin sera showed a weak but reproducible precipitation with spectrins purified from sheep or rat erythrocytes. This cross reactivity allowed use to use the anti-human spectrin sera also for the estimation of spectrin in different tissue culture cells of rat origin. In this system the heterologous antisera must be fivefold more concentrated to give comparable binding. The sensitivity in these conditions allows the estimation of about 50–100 ng of rat spectrin per ml In the homogenates of four different rat cell lines studied (2 cloned lines of SV40 transformed embryo fibroblasts, the glial cell clone 6, and a line derived from a mammary

Table 1 Reaction of crude cell extracts and homogeneous human spectrin in a radioimmunoassay for spectrin

Disrupted cells and additions	Protein concentration of the assayed extract (mg ml^{-1})	^{125}I-spectrin (human) bound (% of input)	Amount of spectrin potentially present (% of extracted protein)
None	—	40.5	—
Human			
Erythrocytes	1.6×10^{-2}	18.3	2.4×10^{-1}
HeLa*	10.0	38.5	$<4\times10^{-5}$
HeLa†	13.5	36.4	$<4\times10^{-5}$
WI 38	9.4	38.8	$<4\times10^{-5}$
SV 80	9.1	37.0	$<5\times10^{-5}$
Spectrin	2.5×10^{-5}	22.5	100
HeLa† and Spectrin	13.5 and 2.5×10^{-5}	23.8	
Monkey			
Vero	9.8	40.1	$<1\times10^{-5}$
CV 1	9.8	36.3	$<6\times10^{-5}$

Washed cells were disrupted at ice-bath temperature by 30 strokes in a tight fitting homogeniser The buffer used contained 30 mM Tris-HCl, pH 8.0, 1% Triton X-100, 0.1 mM EDTA and 0.1 mM PMSF. After 30 min on ice, unsolubilised material was removed by centrifugation (20,000g, 15 min, 4 °C) and 0.2-ml aliquots of the supernatants were tested, in parallel with purified spectrin as standard in conditions as described in Fig. 1. Solubilisation of erythrocytes was almost complete Examination of the small sediment by gel electrophoresis in SDS showed only traces of the characteristic spectrin doublet indicating that much less than 10% of the spectrin remained unextracted This was also indicated by the results of the radioimmunoassay (see text). Protein was assayed by a modification of Lowry's procedure[19]

* Grown as monolayer

† Grown in suspension

adenocarcinoma) no spectrin could be detected at a level higher than $3 \times 10^{-3}\%$ of the extracted protein

In summary, it seems that erythrocyte-type spectrin is not an ubiquitous protein, although we can not exclude the unlikely possibility that non-erythrocyte cells contain spectrin(s) which are immunologically unrelated to erythrocyte spectrin Spectrin is probably an important protein of the erythrocyte—and perhaps some yet unidentified cells—and probably has an important role for the special membrane of this highly differentiated cell.

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Precocious development of foetal glucuronidating enzymes induced by glucocorticoids in culture and *in utero*

CONJUGATION with glucuronic acid is the major pathway in vertebrates by which harmful organic compounds are detoxicated. It ensures the excretion of excess endogenous compounds such as steroid hormones, catecholamines and bilirubin, and of a great range of toxic xenobiotics such as drugs, pesticides, industrial pollutants and their metabolites[1]. Glucuronidation, however, is virtually absent in the early mammalian foetus and approaches adult levels only in the perinatal period, putting the foetus and newborn at considerable risk from unwise medication or exposure to environmental contaminants. This susceptibility has been demonstrated frequently, often with fatal results[2,3]. Defective glucuronidation of endogenous bilirubin is a major cause of human neonatal jaundice and can lead to infant death[4]. Attempts have been made to induce the responsible microsomal enzyme(s), UDP-glucuronyltransferase (EC 2.4 1.17), in cases of prematurity, genetically defective enzyme formation or poisoning by glucuronidogenic compounds; administration of xenobiotic inducers such as phenobarbital has had limited success in neonates and the term foetus[2-5]. Adequate treatment of such conditions, however, requires knowledge of endogenous compounds which induce the enzyme perinatally. We report here the first evidence in foetal mammalian tissue of precocious development of UDP-glucuronyltransferase activity and of overall glucuronidation brought about by known compounds of endogenous nature. These findings provide insight into the perinatal development of this important enzyme.

We have already established that glucocorticoids invoke precocious development of the transferase in chick embryo liver. As that work involved pituitary grafting and continuous infusion of hormone *in ovo*, its extension to the mammalian foetus required simpler techniques. Operative stress to the mother, resulting in excessive corticosteroid production, would make controls difficult. We have therefore adopted two approaches: exposing the foetal liver to glucocorticoids in culture and *in utero* by simple maternal injection.

Culture methods were as described previously[6]. Exposure *in utero* was achieved by injecting pregnant rats intraperitoneally on days 14 and 15 of gestation with the steroid dissolved in arachis oil; control pregnant rats received arachis oil only. UDP-glucuronyltransferase activity towards *o*-aminophenol was assayed as described previously[6] but, because the mammalian liver enzyme is markedly activable and the activation is age dependent, assays were carried out in each experiment over a range of digitonin concentrations (0–0.2% w/v) covering zero to beyond optimal activation. Overall glucuronidation, for which the intact cells synthesise their own UDP-glucuronic acid[1], was measured in sliced tissue, as previously[6].

We found transferase activity towards *o*-aminophenol in the naturally developing rat foetus to be virtually zero from days 14–17 of gestation, increasing sharply on day 17 to peak on day 20. This held for all degrees of activation of the enzyme studied. We then exposed 14-d rat foetal liver explants to 2 μM corticosterone or cortisol in culture in a protein-free defined medium After 3 d in culture transferase activities had reached values normally found in 18–19-d foetal liver *in utero*, almost the 'adult' levels. Dexamethasone, 2 μM in the culture medium, increased the values to full adult male levels. 'Control' 14-d foetal livers, left for 3 d *in utero*, possessed negligible levels, as did those cultured without the added glucocorticoid (Table 1).

Pulsing the glucocorticoid-exposed cultures for 5 h with 3.5 μM cycloheximide inhibited ^{14}C-leucine uptake and prevented development of transferase activity; both processes returned within 2 d to the rates observed in non-pulsed cultures. The data also indicated that general protein synthesis proceeded at similar rates in cultured liver whether exposed to glucocorticoids or not; further, after culturing for 2 d without glucocorticoids, subsequent exposure to them evoked transferase production as rapidly as in fresh cultures. The glucocorticoids were thus not needed to maintain viability of the cultured tissue but seemed responsible for the specific increase in transferase. Other steroids (not glucocorticoids), exhibited no effect.

Table 1 Development of UDP-glucuronyltransferase activity in foetal rat liver exposed to glucocorticoids

Treatment of 14-d foetal liver sample	nmol *o*-aminophenyl glucuronide formed per mg protein per h	
	In culture	*In utero*
Fresh (untreated)	—	<2.0
After 3-d control treatment	2.8, 2.2	2.4, 2.7
After 3-d cortisol treatment	25.8, 25.0	7.0, 7.6
After 3-d dexamethasone treatment	45.3±9.8 (4)	28.4, 30.8

Liver fragments were cultured on rafts over Eagles' MEM supplemented with L-glutamine and penicillin–streptomycin mixture[6] and containing 2 μM steroid where required. Pregnant rats were injected intraperitoneally with 8 mg cortisol or 0 3 mg dexamethasone in arachis oil per day on the days 14 and 15 of gestation. Control rats received arachis oil only; liver transferase levels in control foetuses were identical with those in foetuses from uninjected mothers. Adult male rat transferase levels were 30–40 of above units. Transferase activities were assayed[6] in 'optimal' activation conditions (see text). Results above are from typical experiments. Each result is from a pool of at least 4 foetal livers; where appropriate, s.e.m. is given, with number of experiments in parentheses.

Table 1 also shows the effect on foetal liver transferase of maternal injection of glucocorticoids. As glucocorticoids cross the rat placenta[7], and dexamethasone is less likely to be inactivated by the mother[8], the precocious development of transferase activity following injection of cortisol or dexamethasone presumably reflects the action of these compounds on the foetal liver *in utero* as described above during culture. Transferase stimulation was found to be linearly dose dependent.

Overall glucuronidation, as measured in foetal liver slices or explants, also precociously increased after exposure to the glucocorticoids in culture or *in utero* We have induced precocious transferase activity to several substrates, endogenous and xenobiotic, this way but stimulation towards others, apparently those developing later in the perinatal period, have so far resisted our efforts both in culture and *in utero*.

We have thus reported the first evidence in foetal mammalian tissue of precocious development of UDP-glucuronyltransferase activity and of overall glucuronidation brought about by known compounds of endogenous nature. These compounds are glucocorticoids. Their effect is reproduced by the long-acting synthetic glucocorticoid, dexamethasone. They evoke high transferase activity in foetal rat liver from negligible levels, both in culture and *in utero*. They act directly on the liver, and require protein synthesis for their precocious induction of the transferase. Their involvement perinatally is consistent with the peak in corticosterone levels observed in foetal rat plasma on gestational day 19 and with the maternal increase on day 18 (refs. 9–11) We therefore suggest that the natural perinatal development of glucuronidation and UDP-glucuronyltransferase activity towards certain substrates is brought about by glucocorticoids

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Liver protein metabolism response to cold in genetically obese (*ob*/*ob*) mice

THE response of obese (*ob*/*ob*) mice to the cold is reportedly impaired to the extent that they die 3–6 h after exposure. They also increase their oxygen consumption to a lesser extent than do their lean littermates in response to the cold[1,2] As *ob*/*ob* mice have increased insulation due to excess body fat and respond in this way to the cold before the phenotypic expression of obesity, impaired thermogenesis seems to be a primary feature of this strain of genetic obesity Adult obese Zucker rats also show impaired thermogenesis when exposed to cold and their mean survival time is 28 h (ref 2). The nature and control of this thermogenesis and its possible relevance to energy balance is unclear The involvement of protein metabolism and the energy cost of protein turnover has been postulated by[1–6]. Acclimatisation to the cold increases protein turnover in rats[6] and in lean (*Ob*/+) mice (unpublished observations).

We report here a comparison of rates of amino acid incorporation into liver protein by lean (*Ob*/+) and obese (*ob*/*ob*) mice. Amino acid incorporation was depressed in the obese mice, in response to a cold environment when compared to their lean littermates.

Female lean and obese mice, age-matched (70±5 d) were kept at 22 °C and fed freely until required. Animals were then placed in a 4 °C environment with access to food, and killed in groups of four, from zero time to 24 h after cold exposure. The length of the comparative study was

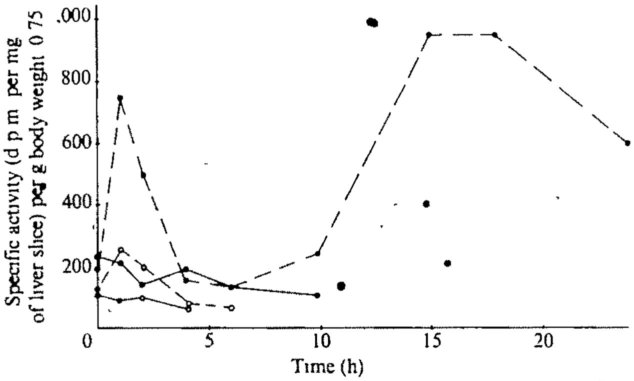
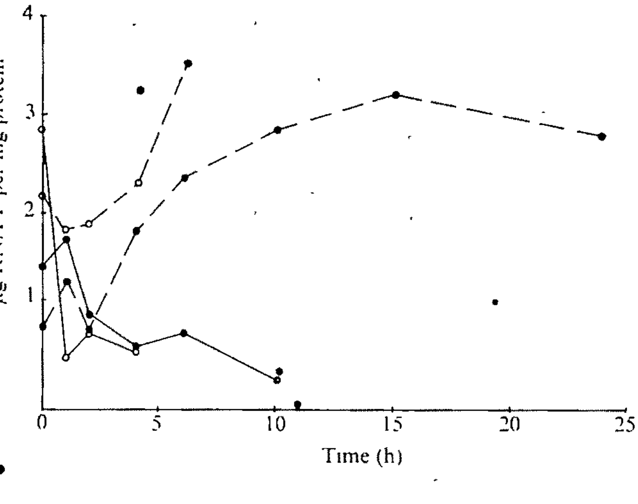


Fig. 1 Changes in liver amino acid incorporation following exposure to 4 °C in lean and obese mice with and without food. ○——○, obese without food, ○ - -·- ○, obese with food, ●——●, lean without food; ●-·-·-●, lean with food. Animals were killed by cervical dislocation, the livers rapidly removed, placed on a Petri dish packed with ice and three or four slices approximately 1 mm thick were cut with a razor blade The slices from each liver were placed in individual flasks with 2.5 ml of Krebs phosphate Ringer solution (100 parts 0.9% NaCl; 4 parts 1.15% KCl; 3 parts 1.22% $CaCl_2$; 1 part 3.82% $MgSO_4$ 7 H_2O and 20 parts 0.1 M phosphate (Na) buffer *p*H 7.4) The buffer was prepared from 0.1 M $NaH_2PO_4 2H_2O$ (14%) and 0.1 M $Na_2HPO_4 2H_2O$ (86%). 1 μCi of L-4,5-^{3}H-leucine (55 Ci mmol^{-1}) was added, and after oxygenation the slices were incubated at 37 °C for 1 h. The incubation was stopped by removal of the slices, which were blotted and homogenised in TCA (100 g l^{-1}). The precipitate was washed twice with a saturated solution of unlabelled leucine in TCA (100 g l^{-1}) to remove any unincorporated labelled leucine TCA was removed by washing with ether and the precipitate solubilised in 3 ml of 0.3 M KOH. The activity of the protein was measured by liquid scintillation counting and protein determination was by Autoanalyser[8].

Fig. 2 Liver RNA to protein changes following exposure to 4 °C in lean and obese mice with and without food. ○ —— ○, Obese without food; ● —— ●, lean without food; ○ -·- - ○, obese with food; and ● -·- ●, lean with food.



controlled by the survival time of the obese (*ob/ob*). The livers were immediately removed, and amino acid incorporation into protein was measured as described in Fig 1 The rest of the liver was used for RNA determinations[7] and the residual protein pellet was solubilised in 5 ml of 0.3 M KOH for protein determination by Autoanalyser[8].

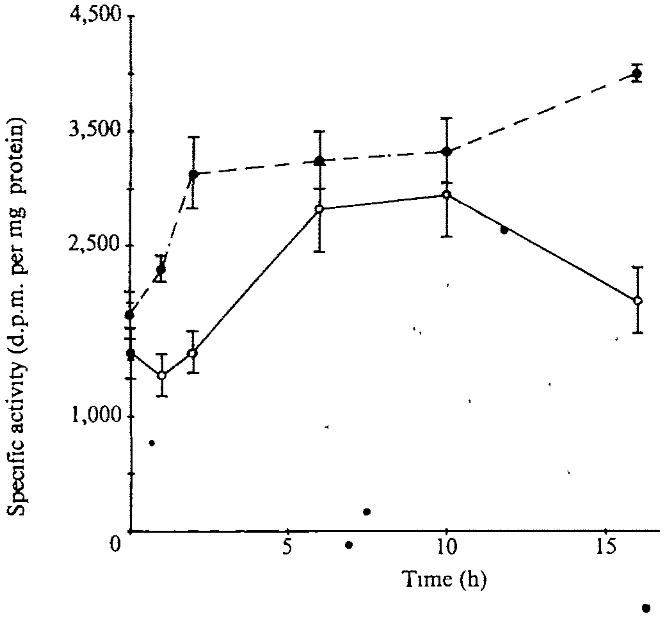
Ratios of RNA to protein in livers from obese mice were significantly greater ($P<0.001$) than those of lean mice at time zero. This was confirmed by monitoring the response to cold without food (Fig. 2). This suggests that there is some restriction on the effective use of RNA species after the transcription stage of protein synthesis in obese mice.

The ratio of RNA to protein was significantly greater in both lean and obese animals after exposure to cold (Fig. 2). But after 4 h, only the ratio for the lean animals was significantly greater ($P<0.02$) than the corresponding ratio measured at time zero

Amino acid incorporation in the lean mice was significantly increased after 1 h of exposure and the percentage increase over zero values was greater in the lean than the obese mice, whether expressed directly as specific activity of liver slice protein or as (Fig. 1) specific activity per unit of body wt$^{0.75}$. The results are expressed as the latter because if protein turnover is contributing to thermogenesis, the ability of the heat produced by the process—to maintain body temperature—must be related to metabolic body size (body weight$^{0.75}$). Obese mice were dead within 8 h and so a second increase in amino acid incorporation, like that found in the lean mice, could not be demonstrated. Either the failure of some other process caused a decrease in body temperature so that the suppression of amino acid incorporation was a secondary effect, or the increase of amino acid incorporation is critical to the maintenance of body temperature. The latter seems more likely as there is a significant difference between lean and obese mice with food and between lean without food after 1 h of cold exposure, when body temperature will not have fallen sufficiently to explain the decrease in amino acid incorporation[2].

The secondary increase in incorporation in the lean animals can be explained by the increase in RNA, whereas the primary increase in incorporation must be controlled by other factors, probably hormonal. But even though RNA levels in the obese mice after 6 h were significantly increased ($P<0.001$) (Fig. 2) there was no associated increase in amino acid incorporation.

Fig. 3 Changes in liver amino acid incorporation following a meal in lean and obese mice ○——○, Obese; ●–·–·–●, lean. Animals were starved for 48 h, and then fed Porton mouse diet (Christopher Hill) for the remainder of the experiment. All animals after 1 h had full stomachs. Amino acid incorporation was measured as in Fig. 2.



Unfed mice were used to find whether the increased food intake, by cold adapted animals[9] and specifically by lean mice (unpublished observations) was essential for adaptation to the cold. In these circumstances neither lean nor obese mice could adapt to the cold, the obese mice died 2 h earlier than in the previous study and the lean animals died after 11 h of exposure. In neither lean nor obese mice was there an increase in amino acid incorporation and the RNA to protein ratio decreased in both groups (Figs 1 and 2).

These observations seem to support the hypothesis that food has a thermic effect[10]. But in a study performed at 22 °C when lean and obese animals were starved for 48 h and fed (Figs 3 and 4) there was only a twofold increase in amino acid incorporation, compared with the fivefold increase observed in the cold We therefore suggest that food contributes directly to thermogenesis, but that the large increase observed in the cold cannot be explained entirely in this way There must be simultaneous hormonal stimulus of thermogenesis and amino acid incorporation. No hormonal stimulus was observed in the cold without food presumably because substrate availability was rate limiting.

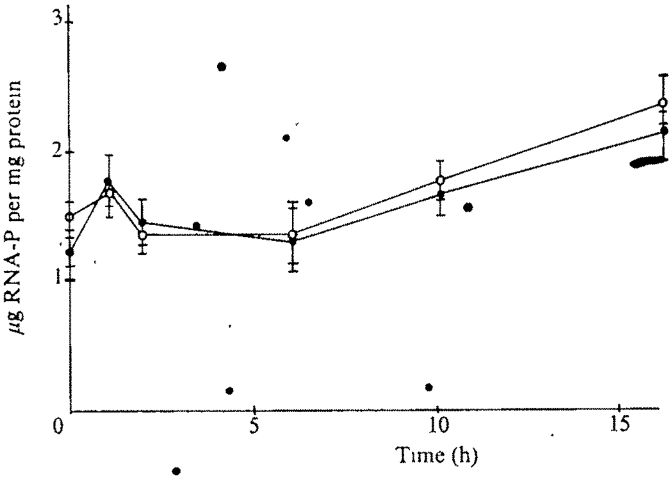


Fig. 4 Liver RNA to protein changes following a meal in lean and obese mice. ○, Obese; ●, lean.

RNA to protein ratios for lean and obese mice were not significantly different for the starved animals, and were only slightly increased after feeding (Fig. 4). The levels in obese mice had decreased to those of lean mice after 48 h of starvation, which agrees with the observation that the former levels decreased to the latter values if mice were exposed to the cold without food. This supports the argument that RNA levels are high in the livers of obese mice because of an impaired protein synthesis. In starvation when protein synthesis is severely restricted due to lack of substrate, the effect of such an impairment which increases RNA would be negated.

In an obese animal placed in the cold without food, shivering thermogenesis would make such a major demand on substrate that protein synthesis would be restricted in a similar manner. Amino acid incorporation in the obese in response to food (Fig. 3) was depressed in comparison with the lean and was not so prolonged, which may affect energy balance by depressing energy expenditure thus contributing to the obesity.

The difference between amino acid incorporation in the livers of lean and obese mice is not the only consequence of exposure to the cold Jansky[11] has shown in cold-adapted rats that the liver only contributes 25% of non-shivering thermogenesis (NST), the remainder coming from muscle (50%), digestive tract (10%), brown fat (10%) and other tissues (5%).

It is not known whether protein metabolism in these tissues can be implicated in NST Also, the involvement of futile cycles in thermogenesis has been postulated[12]. Specifically, James and Trayhurn[13] have implicated a futile cycle in glucose metabolism found in skeletal muscle, the impairment of which in obese subjects reduces their ability to produce heat, either in a cold environment or after food. They state that[13], "The control and interrelationships between different futile cycles are at present unclear".

Bray *et al.*[14] demonstrated that exposure to the cold does not lower the body temperature of obese (*ob*/*ob*) mice if they are pretreated with triiodthyronine, and that the body temperature of lean mice decreases if they have been made hypothyroid by prefeeding with propylthiouracil. This suggests thyroid hormones as likely candidates for the hormonal control. Jansky[11] demonstrated that noradrenaline also stimulates thermogenesis, so there are at least two candidates for hormonal control. The relative importance of each is unclear especially as the calorigenic effect of noradrenaline depends on thyroid status[15].

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Contractile activation in slow and twitch muscle fibres of the frog

VERTEBRATE slow and twitch skeletal muscle fibres differ qualitatively in many respects and constitute functionally distinct motor systems[1,2] Little is known, though, of the respective contractile activation properties. Since triad contacts between transverse (T) tubules and sarcoplasmic reticulum (SR), thought to be the pathway for activation of twitch fibres, are nearly identical in frog twitch and slow fibres[3], similar processes would be expected to lead to Ca^{2+} release from the SR following T-tubule depolarisation in both muscle types. We describe here voltage-clamp studies of activating threshold contractions in slow and twitch fibres[4,5]. Activation kinetics in these physiologically very different fibre types are remarkably similar. Thus, a given membrane potential change may result in an equivalent amount of Ca^{2+} delivered to both slow and twitch myofibrils Once activated, each fibre type would shorten at its characteristic velocity[6], probably determined by the rate of actomyosin cross-bridge turnover[7].

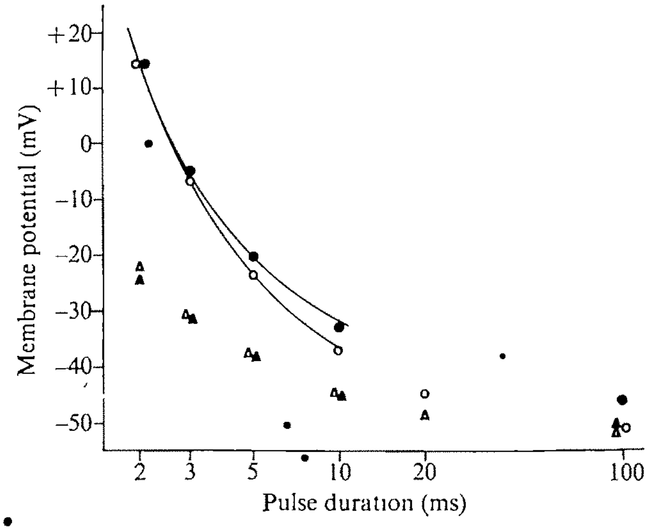
Experiments were performed at 9 °C on sartorius twitch fibres and pyriformis slow fibres of English frogs (*Rana temporaria*). Normal Ringer contained 115 mM NaCl, 2.5 mM KCl, and 1.8 mM $CaCl_2$ buffered at *p*H 7.0 with 1 5 mM Na-phosphate; twitch fibres studied were poisoned with tetrodotoxin (10 μg ml^{-1}).

Generally we used the methods described by Costantin[5]. Membrane potential was measured differentially between an internal microelectrode and another 20–30 μm away outside the fibre Time constants of these electrodes and command pulses to the clamp circuit were matched (~150 μs), and current-passing microelectrodes were shielded with conducting paint and then insulated These procedures facilitated rapid membrane potential changes using high resistance electrodes necessary for the slow fibre experiments[8]. Holding potential was –90 mV throughout.

For a given pulse duration, the threshold membrane potential was taken as that at which discrete sarcomere shortening was visually detected, the region under observation being between the microelectrodes and in the plane of focus through the upper or lower surface of the fibre. For the briefest pulses, a range of up to 5 mV was occasionally required to decide on an unequivocal contraction, threshold was taken as the midpoint of such a span. This was more troublesome in slow fibres due to the low shortening velocity.

In a frog twitch fibre the amplitude of a step depolarisation eliciting a threshold contraction increases markedly as

Fig. 1 Strength–duration curves for twitch (●, ▲) and slow (○, △) muscle fibres. Circles represent means from 14 slow and 18 twitch fibres studied at 9 °C; triangles are from 21 slow and 18 twitch fibres at 20 °C Smooth curves were calculated with the equation[4]: $(V_T+C)t_c = B_T$, where V_T is threshold membrane potential (mV), t_c is pulse duration (ms), and C and B_T are empirical constants determined as described by Costantin[5]. B_T = 127±4.7 mV.ms (s e.m.) and C = 49 1±0 9 mV for slow fibres, B_T = 116±5.6 mV.ms and C = 43 5±1.0 mV for twitch.



pulse duration decreases, resulting in a familiar smooth strength–duration (S–D) relation[4,5]. Such S–D curves for slow and twitch fibres in normal Ringers are shown in Fig. 1. Clearly, at either temperature, the relations for slow and twitch fibres are of the same form. At 9 °C the curves superimpose at short times and diverge to near-rheobase 100-ms values of -51 ± 0.8 mV (s.e m.) in slow fibres and -46 ± 0.8 mV in twitch. At room temperature the curves for both fibre types are indistinguishable.

Although the S–D curve reflects events leading to Ca^{2+} activation of thin filaments, it is not clear which processes determine its exact form. During a long pulse, the situation is complicated, since both release and reuptake of Ca^{2+} by the SR will occur, and rheobase represents the steady-state with one process just balancing the other. Uptake of Ca^{2+} during a short pulse and for a few ms afterwards should be negligible[9], so that only processes leading to Ca^{2+} release should fix the requirements a brief depolarisation must satisfy to activate contraction.

One starting point, then, for interpreting the comparison of S–D relations is to assume that for short pulses; a threshold contraction results from release of a particular amount of Ca^{2+}. Rate limiting steps leading to release could occur at the triad, presumably where T-tubule membrane potential is sensed by the SR, or at the sites of Ca^{2+} release themselves. Thus, 2 and 3 ms thresholds at 9 °C should be most indicative of gross differences in the activation kinetics in slow and twitch fibres, but such differences are not seen.

Another similar experiment involves studying the temporal summation of two sub-threshold depolarisations in activating a contraction[4,5]. This yields information on the time course of decay in 'mechanical excitability' induced by the first pulse The fraction of excitability remaining at any time after the first pulse can be estimated by comparing amplitudes of a second test pulse (V_2) eliciting a threshold contraction and of a single threshold pulse of identical duration (V_T; see legend and insert in Fig 2) To maximise the voltage range covered by V_2, brief first pulses (80–90% of threshold) were followed with test pulses of identical duration (3 ms in twitch; 5 ms in slow).

Results from one twitch and two slow fibres are shown in Fig 2. In all fibres the level of mechanical excitability decayed rapidly after the first pulse with a half-time of about 1 ms (Fig. 2*a*). All fibres then showed a slower phase of decay, but with vastly different rates in slow and twitch. Twitch fibre excitability decayed with a 5–6-ms time constant, threshold returning to the original V_T value within about 20 ms, whereas the excitability of the slow fibres returned to the normal level with a time constant of more than 300 ms (Fig. 2*b*). No such very slow component was seen in three other twitch fibres studied.

As argued by Costantin[5], the fast initial decay in a twitch fibre probably represents rapid voltage-dependent deactivation of Ca^{2+} release following repolarisation. Since kinetics of mechanical activation, as evidenced by the S–D curves, seem indistinguishable in the two fibre types, the fast decay of excitability should also be alike in both, and this seems to be so.

Most striking are differences in the slow phase of excitability decay. For twitch fibres, this decay is of the proper time course to represent background Ca^{2+} removal by the SR[5,10]. Since a slow fibre has less SR than a twitch fibre[11], the net rate of Ca^{2+} removal may also be lower, and the very slow decay might then also represent Ca^{2+} uptake. Preliminary experiments measuring tension produced by voltage-clamped short cruralis[12] slow fibres support this idea. After long (1–3 s), just suprathreshold depolarisations, relaxation proceeded with a half time of 0.5–1.0 s.

From these results it seems that the kinetics of mechanical activation in frog slow and twitch fibres are quite alike. Furthermore, buffering the external Ca^{2+} concentration at $< 10^{-8}$M with 2.5 mM EGTA and 10 mM $MgCl_2$ leaves the S–D curves of both fibre types essentially unchanged[13], again suggesting a common mode of activation through release of Ca^{2+} by the SR.

Although activation in an overall sense is kinetically similar, the underlying molecular processes in each fibre type may not be identical. Interestingly, the drug dantrolene sodium, which affects the S–D curve of frog twitch fibres[14], possibly by interfering with Ca^{2+} release[15], has no effect on the S–D relation for slow fibres (unpublished data).

If identical brief depolarisations lead to the same amount of Ca^{2+} released in both fibre types, one might expect, neglecting possible differences in thin filament Ca^{2+} sensitivities, similar numbers of perhaps both SR Ca^{2+} release sites as well as T-tubule : SR triad contacts in both types. Disposition of the internal membranes in slow fibres has been clarified with high voltage eletrcon microscopy[16–18]. Though preliminary, these results suggest that the number of triad (or dyad) 'feet' bridging the T : SR junctional gap in a given volume of muscle fibre may be quite similar for twitch and slow (C. H. Bailey and L. D. Peachey, personal communication). If the feet do subserve an important step in mechanical activation[19,20], similarities in activation kinetics described here may have an anatomical basis, and it may not be necessary to speculate on alternative pathways of activation in frog slow fibres.

We acknowledge the encouragement and support of the late Dr L. L. Costantin and Dr W. K. Chandler. This work

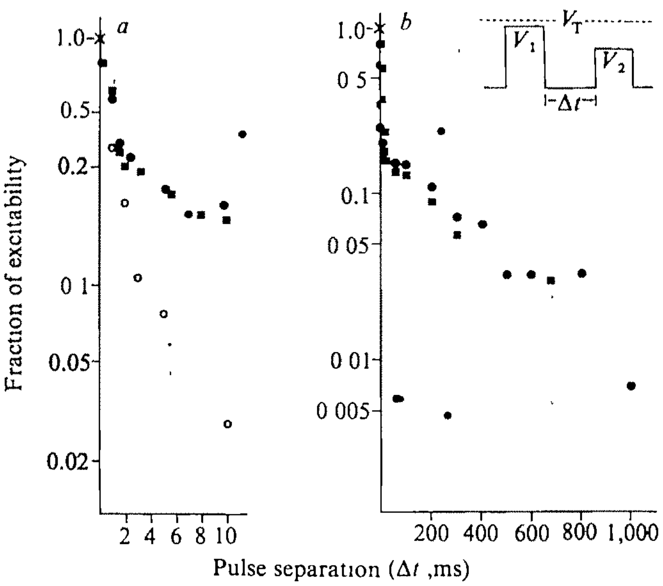


Fig. 2 Decay of mechanical excitability at 9 °C in twitch and slow fibres after a brief subthreshold pulse (V_1). Fractions of excitability remaining at a time Δ*t* were estimated as: $[V_T - V_2(\Delta t)]/[V_T - V_2(0)]$, where $V_2(\Delta t)$ is membrane potential during the V_2 test pulse with pulse separation Δ*t*, and $V_2(0)$ is membrane potential of V_2 with zero pulse separation. V_T is the usual threshold membrane potential for a single pulse of the same duration as V_1. V_T was checked periodically during the experiment and did not change by more than a few mV. V_1 and V_2 pulses were of the same duration throughout: 5 ms in slow and 3 ms in twitch fibres, *a*, Fast excitability decay in one twitch (○) and two slow fibres ●, (■) Cross represents value shared by all fibres. Twitch fibre threshhold returned to normal in about 20 ms. *b*, Slow excitability decay in the two slow fibres of (*a*). Threshold returned to the original V_T level only after 1000–2000 ms.

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Is thyroxine a regulatory signal for neurotubule assembly during brain development?

TUBULIN, the basic subunit of microtubules[1], may be assembled, *in vitro* directly from crude brain supernatants to microtubule-like structures[2,3]. We have used this methodology to measure the initial rate of assembly *in vitro* in crude brain supernatants prepared from rats of different ages. Previous results[4,5] provided clear evidence that at a late foetal stage or just after birth the concentration of tubulin in the brain is not the rate limiting factor for microtubule assembly. We assumed therefore that a factor other than tubulin is necessary to initiate the assembly process and that this factor is limiting in young brain. On the other hand, brain maturation is a slow post-natal process in the rat and it is generally believed that this process is under thyroid hormone control. We report here that microtubule formation during brain development requires the presence of an initiation factor and that the onset of this factor is under thyroid hormone control.

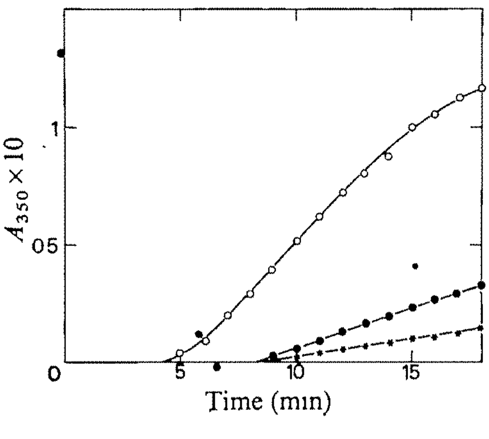
Thyroid hormones have a profound effect on brain development; for example, congenital hypothyroidism produces severe mental retardation (cretinism) in man and animals. Treatment with thyroid hormones after birth accelerates the development of innately organised behaviour and of electrocortical activity[6]. Histological alterations observed during hypothyroidism[7–9] mainly concern the development of cellular processes and dendrites. These effects are associated with biochemical changes; the protein content and protein synthetic activity of the brain are reduced[10,11]. None of these observations, however, is directly related to a possibly more specific effect of thyroid hormones during brain development—the increase in the number of connections, for instance of dendrites spines of cortical neurones and of Purkinje cells in the cerebellum[7].

Microtubules are good markers of brain connections as they are very abundant in these structures[12–14]. At early stages of development, however, tubulin polymerises very poorly *in vitro* in conditions described by Weisenberg[2] and Shelanski[3]. Figure 1 shows, for instance, that in 3-d-old rat brain preparations the rate of *in vitro* microtubule assembly is markedly lower than in those of 15-d-old rats, and also that the lag period is increased; the critical concentration[15] of tubulin at which microtubule assembly can be observed *in vitro* was also increased (not shown). This difference in rates depends on the fact that, at early stages of development, a factor which is needed to initiate the assembly process, is limiting[16,17]. This factor has been tentatively identified[16] with the protein described by Weingarten *et al.* [18]. Another possibility would have been that the young brain supernatants contain an inhibitor of the microtubule assembly; this possibility was eliminated, as reported before[5], by mixing supernatants from 30-d-old and newly born rats: there was no inhibition of assembly in these conditions. In addition, mixing supernatants of newly born rats with very small amounts of sonicated fragments of adult preparations resulted in a normal rate of assembly[5].

Severe hypothyroidism can be produced in the rat by treatment with antithyroid drugs during pregnancy and after birth[7]. Pregnant females were force-fed from the 18th d of gestation until 15 d after delivery with 3-methylthiouracil (MTU); thus MTU was supplied to the foetus during pregnancy and to the newborn rats through the milk of the mother during lactation. At 15 d after birth the newborn rats were clearly hypothyroid—the body weight was 28.8±2.18 g (control rats 34.16±0.3 g). The brain weight was also reduced but only by 20%. The tubulin contents of these supernatants evaluated by a modification[4] of the ^{3}H-colchicine binding method[19] were very similar (controls, 2.87 mg; MTU-treated 2.74 mg tubulin per g wet weight).

In vitro microtubule assembly in brain supernatants was followed using the turbidimetric method of Shelanski *et al.*[3] Figure 1 shows that, after MTU treatment, microtubules polymerised very poorly *in vitro* when the same tubulin concentration was used in the assays (15-d-old MTU treated rat brain supernatants) and in the controls (15-d-old normal rat brain supernatants). The initial rate of assembly markedly decreased and the latency increased. The kinetics of assembly ressembled those observed 3 d after birth, suggesting that hypothyroidism produces a retardation or a blockage of the ability of tubulin to assemble to microtubules. These results suggest that thyroid hormones have a greater effect on the

Fig. 1 Turbidimetry time course of supernatants from 3 and 15-d-old euthyroid rats and 15-d-old hypothyroid rats. The supernatants were obtained after homogenisation of rat brains in a reassembly buffer containing: 0.1 M morpholino-ethane sulphonate (*p*H 6.5, 1 mM GTP, 0.5 mM $MgCl_2$, 1 mM EGTA, 0.1 mM EDTA, 1 mM β-MSH at 4 °C and centrifugation at 105,000*g* for 1 h at 4 °C. Glycerol (final concentration 4 M) was added after centrifugation. Turbidimetry (*A* at 345 nm) time course at 37 °C was followed with supernatants dilutions containing the same (0.940–1.040 mg ml^{-1}) amounts of tubulin, measured as before[12]. 15-d-old hypothyroid rats were obtained by a treatment of the mothers with MTU from the 18th d of gestation (50 mg per day given by force feeding[3]) until the day of killing. ★, 3-d-old euthyroid rat brain supernatant (tubulin 0.94 mg ml^{-1}; ○, 15-d-old ethyroid rat brain supernatant (control) (tubulin 1.05 mg ml^{-1}); ●, 15-d-old hypothyroid rat brain supernatant (tubulin 0.95 mg ml^{-1}).



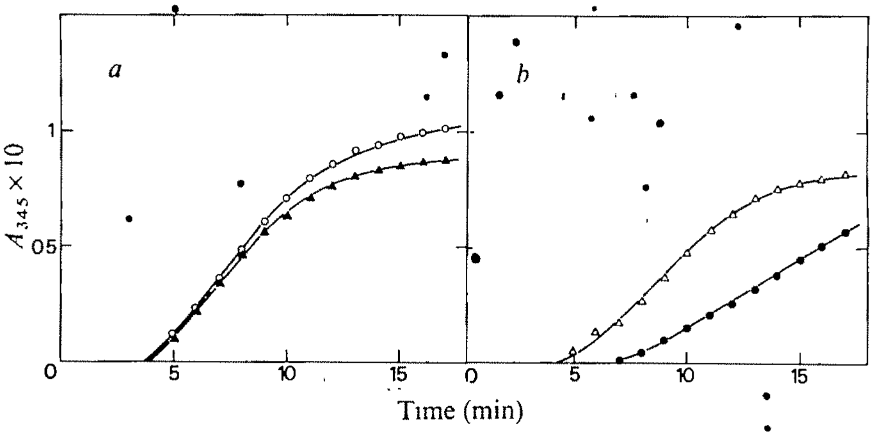


Fig. 2 Effect of thyroxine treatment on the turbidimetry time course of 15-d-old euthyroid and hypothyroid rat brain supernatants (obtained as described in Fig. 1). *a*, ○, 15-d-old euthyroid rat brain supernatant (tubulin 1.04 mg ml^{-1}); ▲, thyroxine-treated 15-d-old rat brain supernatant (tubulin 0.95 mg ml^{-1}). *b*, ●, 15-d-old hypothyroid rat brain supernatants (MTU treated (tubulin 0.95 mg ml^{-1})); △, thyroxine-treated 15-d-old hypothyroid rat brain supernatants (tubulin 1.04 mg ml^{-1}).

initiation process of microtubule assembly than on the tubulin content of the brain.

To test whether the observed low polymerisation was due to the lack of thyroid hormones or to a toxic effect of MTU, normal rats and animals rendered hypothyroid by MTU treatment were injected subcutaneously just after birth with 2 µg DL-thyroxine per day during 6 d and with 4 µg thyroxine per day during the last 9 d before killing. For the hypothyroid rats, thyroxine treatment produced a small increase in the tubulin concentration (MTU + thyroxine 4.04 mg tubulin per g wet weight). Figure 2 shows typical experiments where the effect of thyroxine treatment on the rate of *in vitro* microtubule assembly was studied with identical concentrations of tubulin in the assays and in the controls. Thyroxine treatment had no effect on the control (Fig. 2*a*), whereas the initial rate of assembly was markedly increased and the latency markedly reduced for the MTU-treated rat brain preparations (Fig. 2*b*).

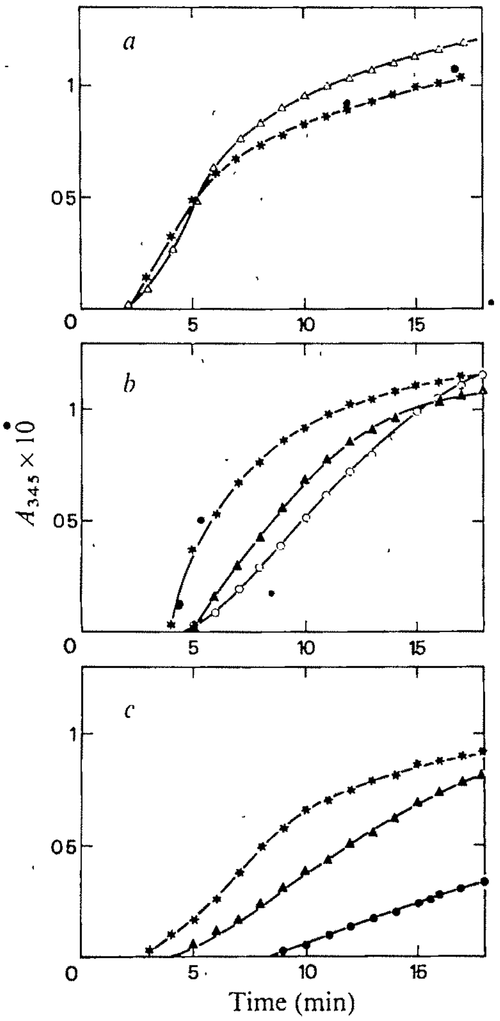


Fig. 3 Effect of τ factor on the turbidimetry (345 nm) time course at 37 °C of adult euthyroid, 15-d-old euthyroid and hypothyroid rat brain supernatants Experimental conditions were as described in Fig 1. τ factor was purified as before[17]. *a*, Adult rat brain supernatant (tubulin 0.88 mg ml^{-1}) in the absence (△) and in the presence (★) of τ factor (0.23 mg ml^{-1}). *b*, 15-d-old euthyroid rat brain supernatants (tubulin 0.78 mg ml^{-1}) in the absence (○) and in the presence of τ factor (▲, 0.115 mg ml^{-1}), (★, 0.23 mg ml^{-1}). *c*, 15-d-old hypothyroid rat brain supernatants (tubulin 0.73 mg ml^{-1}) in the absence (●) and in the presence of τ factor (▲, 0.115 mg ml^{-1}), (★, 0.23 mg ml^{-1}).

The effects of the addition of the τ factor, an initiator of microtubule assembly[18] were also studied. The τ factor was added in increasing amounts to 40- and 15-d-old brain supernatants prepared from euthyroid and hypothyroid rats (Fig. 3). The addition of the τ fraction to the adult supernatant did not produce an increase on the rate of assembly (Fig. 3*a*), suggesting that the adult brain supernatant contains saturating amounts of τ factor. In contrast, addition of τ factor to the 15-d-old brain supernatants produced an increase in rate of assembly (Fig. 3*b*). Thus 15 d after birth the τ factor concentration is still limiting. The effect of the τ fraction on the brain supernatants from MTU-treated rats was even greater; the initial rate of assembly increased to the level of the controls and the lag period decreased (Fig. 3*c*). Thus hypothyroidism probably produces a decrease in the concentration of the τ factor and not in the amount of tubulin. We reported before[18] that addition of a τ preparation significantly increased the rate of microtubule assembly of newly born (3-d-old) rat brain supernatants.

These observations suggest that microtubule formation during normal brain development requires the presence of thyroid hormones. If, as reported by others[20,21], microtubule assembly is required for axon growth and for the formation of nerve connections, the results of this work might contribute to the understanding of the effects of thyroid hormones in brain development and in the aetiology of cretinism.

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Evidence from oxygen exchange measurements for a cooperative interaction between the two heads of myosin

THE present view of the molecular mechanism of muscle contraction[1] does not explain why myosin has two structurally similar globular heads[2]. Each of these heads can interact with actin and with ATP, and each seems to hydrolyse ATP at about the same rate[3]. To investigate possible functional differences between the two heads of myosin, we have compared the rates of intermediate oxygen exchange[4–6] catalysed by heavy meromyosin (HMM), which has two heads, and subfragment 1(S1), which has one head, at different levels of actin activation. This oxygen exchange occurs between water and the γ-phosphoryl groups of two enzyme–nucleoside phosphate intermediates along the pathway of coupled ATP hydrolysis[7]. Thus, measurements of oxygen exchange can be used to probe the kinetics of intermediate steps in the hydrolytic and contractile mechanism.

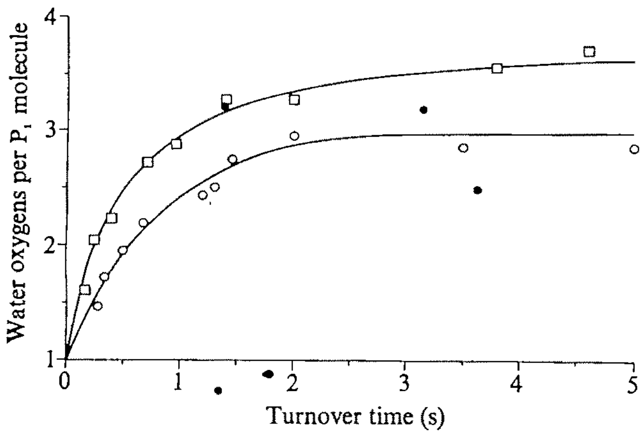

Fig. 1 Incorporation of ^{18}O from water into P_i as a function of turnover time. The assay mixture contained 25 mM KCl, 25 mM Tris buffer (*p*H 7.4), 5 mM $MgCl_2$, 5 mM ATP and appropriate concentrations of proteins in ^{18}O water at 23 °C. S1 was prepared from rabbit myosin by the method of Margossian *et al.*[12] with Mg^{2+} present instead of EDTA to preserve the DTNB light chain. HMM was prepared by the method of Holt and Lowey[13]. Actin was prepared from acetone-dried muscle powder by the method of Spudich *et al.*[14]. P_i was isolated as KH_2PO_4 essentially by the method of Dempsey *et al.*[15]. The ^{18}O content of P_i was determined by the method of Boyer and Bryan[16]. At each concentration of actin, the rate of hydrolysis was obtained from the slope of a linear rate curve and converted to a turnover number (molecules of ATP hydrolysed per second per active site). The turnover time on the abscissa is the reciprocal of this number. Actin markedly activated both fragments up to more than 100-fold at the highest levels. In the conditions of these experiments, with either fragment, the turnover rate increased linearly with the actin level; falling below the line only slightly at the highest level. To obtain a given average turnover time per head, about the same concentration of actin was needed with HMM as with S1. Turnover times were calculated using the equivalent weights of 120,000 per head for S1 (□) and 170,000 per head for HMM (○).

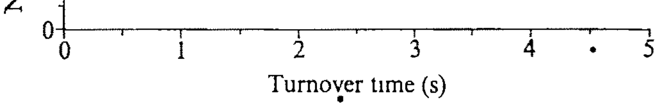

Fig. 2 Calculated number of exchange reactions as a function of turnover time. Conditions are given in Fig. 1 The number of exchange reactions was calculated using the equation of Wolcott and Boyer[8]

$$\frac{\text{water oxygens}}{P_i\text{ molecule}} = \sum_{n=1}^{n} (3/4)^{n-1}$$

where *n* is the number of exchange reactions. In each curve the initial slope, which extrapolates back to one exchange (the one added by the first cleavage of bound ATP) gives the average rate of exchange for the three exchangeable oxygens per P_i molecule. The upper shallow slope estimates the average rate of exchange after two oxygens per P_i molecule have been labelled, that is, when their exchange no longer has any significant or measurable effect on ^{18}O incorporation. Thus, the initial slope minus the final slope is the rate of exchange for two faster exchanging oxygens and the upper slope for one that exchanges slowly. ○, HMM; □, S1.

Any difference in the rate of oxygen exchange indicates either a difference in the lifetimes of the exchanging intermediates, or in the kinetic constants of the exchange reaction itself[4–6,8].

The results reported here show that HMM catalysed approximately half as much oxygen exchange per second per head as did S1. This indicates that in HMM there may be some cooperative interaction between the heads which effectively inhibits some part of the exchange mechanism. Since oxygen exchange is often correlated with the actin-activated ATP hydrolysis coupled to contraction[4–6,9] such an effect could reflect a cooperative action of the two heads in the generation of force or the regulation of contraction.

Actin decreases the turnover time of ATP hydrolysis by myosin and its fragments. It does this by activating the rate-limiting step in the hydrolytic pathway. This rate-limiting step follows oxygen exchange[1,7]. Therefore, the greater the actin concentration, the shorter the turnover time, and the shorter the lifetime of the exchanging intermediates. Figure 1 shows how the incorporation of ^{18}O from water into P_i changed when the turnover time was changed by the concentration of actin in the reaction solution. Figure 2 shows the calculated number of exchange reactions as a function of turnover time. The method for calculating the number of the reactions from the measured incorporation is given in Fig. 2. The slope of each curve in Fig. 2 gives an estimate for the average rate of oxygen exchange, that is, the number of exchanges per second of turnover time.

The use of such plots to study the kinetics of oxygen exchange is described in our recent paper[10]. From the earlier experiments with S1 (ref. 10) we concluded that the four oxygens in each γ-phosphoryl group do not all exchange at the same rate. To summarise our interpretation: the first oxygen is incorporated at virtually zero time by the very fast initial cleavage of the γ-phosphoryl group of bound

ATP (ref. 11); two oxygens exchange at a relatively fast rate by the mechanism of Bagshaw and Trentham[11], that is, by a cycle that first reverses the cleavage of the nucleotide and then recleaves it; each such cycle can cause the exchange of an oxygen atom, if the oxygens can rotate in space so that the one added by cleavage may be different from the one lost by reverse cleavage; the fourth oxygen exchanges very slowly, and this slow exchange indicates that one oxygen may be severely restricted from entering into the exchange cycle by its binding to the protein.

By comparing the slopes in Figs 1 and 2 it became evident to us that HMM, compared with S1, catalysed oxygen exchange at about half the rate per head. In general there are several alternatives for interpreting this finding but one that may have considerable significance to muscle contraction is the clear possibility that only one head of HMM at a time is in a conformation that can catalyse oxygen exchange. This would effectively reduce by half the measured rate of exchange per head. Such a cooperative effect that holds each head in a somewhat different conformation, that is, it does not allow them to take the same conformation at the same time, may coordinate the working of the two heads in the contraction and relaxation of muscle. Work is continuing with other fragments and other forms of S1 to test further whether the marked difference between the oxygen exchange rates of HMM and S1 heads reported here reflects a functionally important property of myosin.

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Haemin protects Met-tRNA$_f$ binding activity of isolated reticulocyte ribosomes from inactivation by protein kinase

RETICULOCYTE lysates incubated in the absence of haemin stop synthesising proteins after a few minutes due to the accumulation of a translational inhibitor (TI) which blocks initiation of protein synthesis[1-3]. This block is characterised by a depletion of initiator tRNA (Met-tRNA$_f$) bound to 40S ribosomal subunits[4,5]. It has been reported that protein kinase activity is associated with TI which has been partially purified from reticulocyte lysates[6]. Addition of the initiation factor eIF-2 (previously designated IF-E2 or IF-MP), which binds Met-tRNA$_f$ and GTP, can overcome the block in initiation which develops in reticulocyte lysates incubated in the absence of haemin or with added TI[7,8]. Phosphorylation of eIF-2 by a protein kinase present in TI preparations has been demonstrated (refs 9–12 and P. Farrel, K. Balkow, R. Jackson and T. Hunt, personal communication), and it has been proposed that phosphorylated eIF-2 is inactive[8,9]. We have investigated the mechanism by which haemin stabilises initiation of protein synthesis by examining the ability of TI to inhibit binding of Met-tRNA$_f$ to ribosomes obtained from reticulocyte lysates supplemented with haemin or from unsupplemented lysates, and the corresponding pattern of ribosome-associated proteins phosphorylated by the protein kinase(s) present in the TI preparation. We have established a correlation between the phosphorylation of proteins associated with native 40S ribosomal subunits and the loss of binding activity of the ribosomes.

TI was prepared from unsupplemented reticulocyte lysates, as described before[6]. Protein synthesis by reticulocyte lysates supplemented with 50 μM haemin is inhibited 80% by 25 μg of TI preparation in a 50-μl assay (unpublished work of Sarma, M. and Baglioni, C.). Ribosomes isolated from reticulocyte lysates (Fig. 1) were incubated with (^{3}H)Met-tRNA$_f$ and GTP. The amount of initiator tRNA bound was measured by passing the incubation mixture through nitrocellulose filters directly or by fractionating the incubation mixture on gradients[13]. Gradient fractions were passed through filters, and Met-tRNA$_f$ was found bound to ribosomes only (data not shown). This binding is GTP dependent and eIF-2 is the only initiation factor reported to bind Met-tRNA$_f$ in a GTP-dependent fashion[14].

Inhibition of Met-tRNA$_f$ binding by TI was assayed by

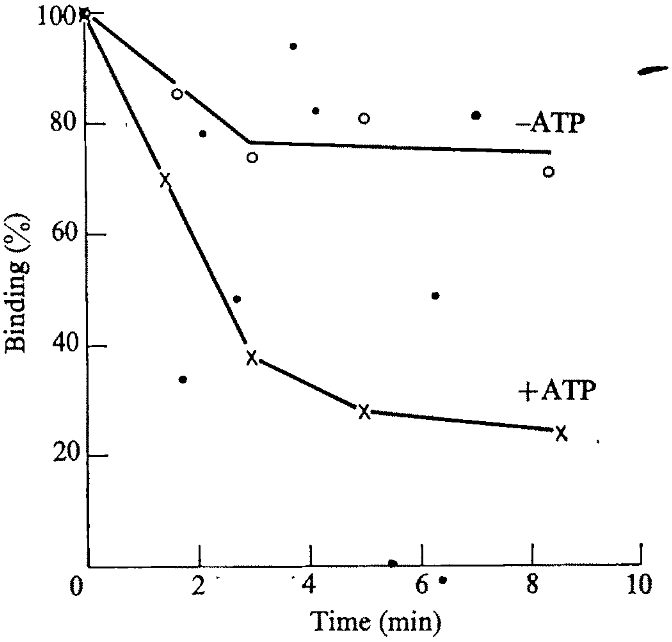

Fig. 1 Time course of inhibition of (^{3}H)Met-tRNA$_f$ binding to ribosomes by a translational inhibitor (TI) in the presence or absence of ATP Reticulocytes were obtained from rabbits made anaemic with 1-acetyl-2-phenylhydrazine. TI was prepared from the postribosomal supernatant of unincubated reticulocyte lysates, following the procedure of Levin *et al.*[6]. The fraction eluted from DEAE-cellulose at 0.24 M KCl was used. Ribosomes were prepared as previously described[14], except that all solutions contained 1 mM dithiothreitol. Stripped Met-tRNA$_f$ from rabbit liver (Grand Island Biological Co.) was charged with (^{3}H) methionine (3.7 Ci mmol^{-1}) using an *E. coli* aminoacyl synthetase (Miles). Approximately 40 μg of ribosomes were incubated in 35μl incubation mixtures containing buffer A (110 mM KCl, 20 mM Tris-HCl *p*H 7.5, 5 mM MgCl$_2$, 1.2 mM dithiothreitol), 12 μg of TI and 40 μM ATP (×) or no ATP (○). After incubation at 30 °C for the time indicated on the absissa 3 pmol of (^{3}H)Met-tRNA$_f$ (7,500 c.p.m.) and 1 mM GTP in 15 μl of buffer A were added and the incubation continued for 5 min at 24 °C. The reactions were stopped and the (^{3}H)Met-tRNA$_f$ bound determined by the nitrocellulose filter assay previously described[14] Samples which were not incubated at 30 °C before the addition of Met-tRNA$_f$ bind about 0.3 pmol of Met-tRNA$_f$. Binding is expressed as the percentage of binding activity relative to such samples.

pre-incubating ribosomes with TI for various times, then adding Met-tRNA$_f$ and GTP (Fig. 1). Only some loss of binding activity is observed on incubation of ribosomes with ATP or TI (Table 1). Addition of 40 μM ATP together with 12 μg of TI however, results in 75% inhibition of the binding of Met-tRNA$_f$ (Fig. 1). We have, therefore, shown an ATP requirement for the inhibition, suggesting that the protein kinase activity of TI is directly responsible for the inhibition of Met-tRNA$_f$ binding to ribosomes in the assay described. The ATP requirement can be shown because ribosomes have been isolated through a sucrose cushion, thus removing endogenous ATP. Incomplete removal of ATP may be responsible for the slight loss of Met-tRNA$_f$ binding activity observed in incubations with no added ATP (Table 1). Addition of 1.5 μM cAMP has no effect on the loss of Met-tRNA$_f$ binding activity (data not shown), indicating that the inhibitor protein kinase is not stimulated by cyclic AMP.

Inhibition of Met-tRNA$_f$ binding by TI and 40 μM ATP is incomplete, leaving about 25% of the original activity of control ribosomes (Fig. 1). This residual binding may be due to eIF-2 activity which is resistant to inactivation by TI A higher resistance to inactivation by TI is indeed observed with ribosomes prepared from lysates supplemented with haemin (Fig. 2). Loss of Met-tRNA$_f$ binding activity is consistently less for these ribosomes than for ribosomes prepared from unsupplemented lysates (Table 1).

Since inactivation of Met-tRNA$_f$ binding in the presence of TI is ATP-dependent and protein kinase activity is present in TI preparations, it is reasonable to propose that specific initiation factor(s) involved in the binding of initiator tRNA to ribosomes are inactivated by phosphorylation. We have, therefore, used (^{32}P-γ)ATP to phosphorylate reticulocyte ribosomal proteins and have examined the proteins by electrophoresis on polyacrylamide gels. Figure 3 shows the autoradiographic pattern obtained when ribosomes are incubated with TI in conditions which lead to the inhibition of Met-tRNA$_f$ binding (track 1). Many of the bands correspond to polypeptides present in the TI preparation (track 5). Notable among the phosphorylated polypeptides of ribosomes (track 1) are those of MW 67,000, 53,000 and 38,000. The 67,000 polypeptide has a MW similar to that of initiation factor IF-M1, which has been reported to be 65,000 (ref. 15). The other bands may correspond to two of the three polypeptides comprising eIF-2.

Table 1 ATP dependence of the inhibition of Met-tRNA$_f$ binding by a translational inhibitor (TI) and protection from inactivation by haemin

Exp. no.	Inhibition (%) +ATP	 +TI	 +TI +ATP	 +TI +ATP +haemin
1	21	19	71	43
2	30	—	73	51
3	24	—	78	—
4	—	11	54	—
5	—	9	64	—
6	—	—	61	50

Ribosomes were incubated 5 min at 30 °C with 40μM ATP, where indicated, and/or 12 μg TI, and then with (^{3}H)Met-tRNA$_f$ and GTP as indicated in Fig 1. Determination of the bound Met-tRNA$_f$ was as described in the legend of Fig. 1. Ribosomes from the incubations designated as '+haemin' were obtained from reticulocyte lysates supplemented with 50μM haemin, all other incubations contained ribosomes from unsupplemented lysates. Inhibition is expressed as the percentage of binding activity lost relative to samples not incubated at 30 °C before addition of Met-tRNA$_f$ and GTP. Different preparations of ribosomes obtained from different lysates were used for each experiment. Addition of TI and ATP after incubation of ribosomes with Met-tRNA$_f$ and GTP does not decrease the amount of bound Met-tRNA$_f$. Protein synthesis in cell lysates is 50% inhibited by 10μg of TI in a 50-μl assay[13], and Met-tRNA$_f$ binding is inhibited 50% by 5 μg of TI in the assay described in the legend of Fig. 1.

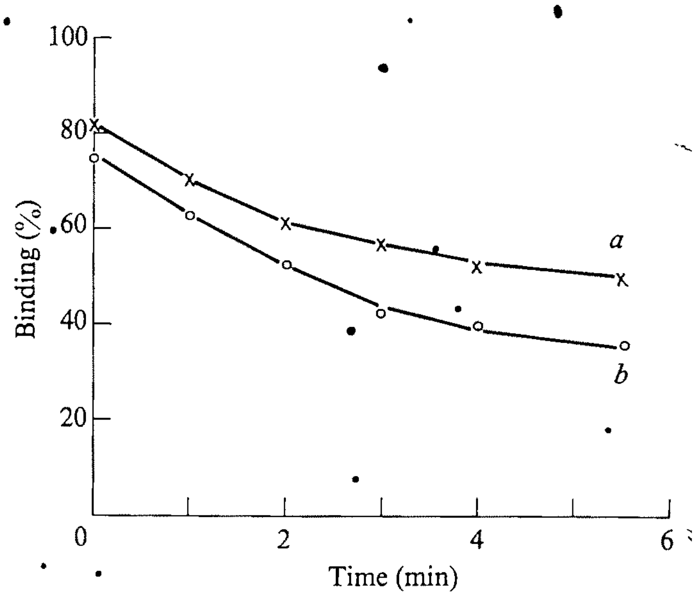

Fig. 2 Time course of haemin protection of (^{3}H)Met-tRNA$_f$ binding to ribosomes from inactivation by TI. Approximately 40μg of reticulocyte ribosomes from either lysates supplemented with 50 μM haemin (*a*) or unsupplemented lysates (*b*) were incubated with 40 μM ATP and TI at 30 °C for the time indicated on the absissa as described in Fig. 1 Then (^{3}H)Met-tRNA$_f$ and GTP were added, the incubation continued at 24 °C for 5 min, and the samples processed as described in Fig. 1. Binding is expressed as a percentage of the amount of (^{3}H)Met-tRNA$_f$ bound relative to samples incubated without TI.

Phosphorylation *in vitro* of each of the three subunits of eIF-2 by protein kinases has been reported (refs 9–12, and P. Farrel *et al.*, personal communication). Moreover, phosphorylation of the 38,000 subunit may be responsible for inhibition of initiation of protein synthesis (ref. 11, and P. Farrel *et al.*, personal communication). Phosphorylation of other initiation factors which stabilise the association of Met-tRNA$_f$ with 40S ribosomal subunits[16] may also play a role in the inhibition of Met-tRNA$_f$ binding.

A difference in the pattern of phosphorylated polypeptides is observed between ribosomes prepared from lysates supplemented with haemin (track 2) and those from unsupplemented lysates (track 1). A polypeptide of MW 53,000 is phosphorylated to a much greater extent in the ribosomes from unsupplemented lysates. This polypeptide is associated with native 40S ribosomal subunits. Incubation of these subunits with TI and (^{32}P-γ)ATP results in the phosphorylation of several polypeptides, among which is the 53,000 polypeptide. Addition of 50 μM haemin to the incubation medium markedly decreases the phosphorylation of this polypeptide (Fig. 3, tracks 3 and 4). The 53,000 MW polypeptide is not associated with salt-washed 40S subunits derived from polysomes (unpublished work of Sarma, M. and Baglioni, C.). Since most eukaryotic initiation factors are associated with native 40S ribosomal sub-units[17,18], it is likely that the polypeptides phosphorylated by TI which are associated with these subunits, but not with salt-washed 40S ribosomal subunits, are initiation factors.

The data presented here show that a certain amount of the Met-tRNA$_f$ binding capacity of ribosomes is resistant to inactivation by TI. Ribosomes from haemin-supplemented lysates show a greater resistance to inactivation by TI than those from unsupplemented lysates. This increased resistance due to haemin is correlated with a decrease in phosphorylation of a specific polypeptide of MW 53,000.

We have established that haemin co-sediments with ribosomes prepared from cell extracts supplemented with 50 μM haemin. By analysing the visible absorption spectrum of these ribosomes, we find the typical spectrum of a haemin–protein complex with a maximum absorption at 390 nm.

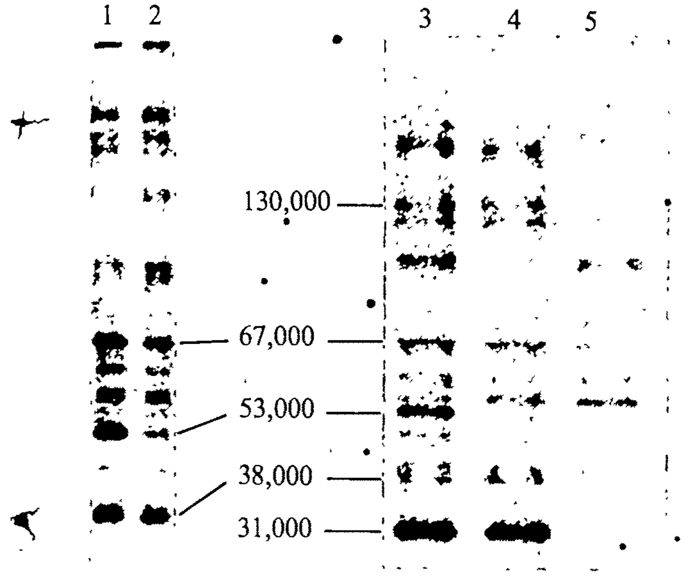

Fig. 3 Polyacrylamide gel electrophoresis of (^{32}P)-labelled ribosomal proteins. For tracks 1 and 2, 40 μg of ribosomes from either lysates supplemented with 50 μM haemin (2) or unsupplemented lysates (1) were incubated with TI as described in Fig. 1, except that 40 μM (^{32}P-γ)ATP (15 Ci mmol^{-1}) and 1.5 μM cyclic AMP but no (^{3}H)Met-tRNA$_f$ or GTP were added. Omission of cyclic AMP results in no change in the pattern of phosphorylated proteins at this ATP concentration. For tracks 3, 4 and 5, 25 μg of TI were incubated with 30 μg of BSA, 400 μM ATP, 5 μCi of (^{32}P-γ)ATP, 1.5 μM cyclic AMP, 25 mM HEPES-KOH (*p*H 7.2), 6 mM Mg(OAc)$_2$, 20 mM KCl, and the following additions: 3, 16 μg of native 40S ribosomal subunits prepared according to Freienstein and Blobel[18]; 4, 16 μg of native 40S subunits and 50 μM haemin; 5, no additions. Incubations were at 30 °C for 30 min in a 50-μl volume. Omission of cyclic AMP resulted in a significant decrease in the phosphorylation of only two bands, one of MW 31,000 which is a ribosomal protein present in salt-washed 40S ribosomal subunits[12] and the other of approximate MW 130,000. Reactions were stopped by adding an equal volume of 2× electrophoresis buffer. Samples were fractionated by electrophoresis on 8-cm gel slabs, made with 12.5% acrylamide and 0.1% *bis*-acrylamide at *p*H 8.7, with a 2-cm, 5% acrylamide, 0.13% *bis*-acrylamide stacking gel at *p*H 6.8 according to Laemmli[21] with *bis*-acrylamide concentrations from Blattler *et al.*[22]. Electrophoresis was at room temperature for 1 h at 50 V, then for 3.5 h at 150 V. Gels were stained with Coomassie Brilliant Blue, dried, and autoradiographed for 1–3 days using Kodak RP Royal X-Omat film. The MW included have been calculated by running in separate tracks marker polypeptides of known MW.

Ribosomes obtained from cell extracts unsupplemented with haemin do not show such an absorption at 390 nm. Haemin may (i) render the 53,000 MW polypeptide a less accessible substrate for a protein kinase, (ii) increase the activity of a phosphatase specific for this polypeptide, or (iii) inhibit a kinase which specifically phosphorylates this polypeptide Haemin may modulate the activity of a protein kinase and of a phosphatase, as suggested by Traugh[19]. The finding that haemin promotes the aggregation of inactive low MW initiation factors into active high MW complexes[20], suggests, however, that haemin may directly change the conformation of initiation factors and possibly render them more resistant to inactivation by protein kinases.

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Does 5S RNA function by a switch between two secondary structures?

THE structure of 5S RNA has been a lively field for experiment and speculation, with no definitive resolution of the problem in sight (see refs 1 and 2 for reviews). Fox and Woese[3] suggested comparative sequence analysis of prokaryotic 5S RNAs as a method for locating base paired regions, an appealing procedure because of its similarity to the method used to substantiate the cloverleaf model for tRNA. Its logic states that if all known 5S RNA sequences contain complementary bases at a site, a double helix probably exists there in the functional form. We propose that comparative sequence analysis suggests two different functional structures for prokaryotic 5S RNA. One of them has been introduced by Fox and Woese[3], and its secondary structure is based on four conserved helices: the 'molecular stalk' (*Escherichia coli* residues 1–10 and 119–110), the weak 'tuned helix' (18–23/65–60), the 'common arm base' (31–34/51–48) and the 'prokaryotic loop' (82-86/94-90). The base paired structure that results is consistent with enzymatic digestion[14] and chemical modification[1,5] results on native 5S RNA, if allowance is made for protection of residues by tertiary bonding as well as secondary structure. This 'model', however, does not explain the apparent conservation of a double helix (33–39/88–82 in *E. coli*) (Table 1) which is sterically inconsistent with two of the helices listed (the common arm base and the prokaryotic loop). Fox and Woese[3] noted this conservation but considered it unimportant because of the variable helix length and the conflict with the other two helices.

Our proposal begins with the observation that the alternate conserved helix explains the enzymatic degradation pattern[4] of the 'denatured' or B form of 5S RNA (ref. 13), whereas the Fox and Woese model does not. Hence the alternate conserved helix, which we term the 'central helix', may be part of the secondary structure of the B form. Since the central helix is conserved over all known sequences, we conclude that it is functionally significant. Therefore the finding of two conformational forms of 5S RNA is neither accident nor artefact, but an indication of an important conformational balance which allows rearrangement of 5S RNA secondary structure during protein biosynthesis. The fact that only the A form has yet been isolated from native ribosomes may be because of instability of the B form except in the elongation factor G/GTP/ribosome complex.

Figure 1 shows the two models. The crucial observations that distinguish them derive from the nuclease digestion experiments of Jordan[4]. He found a fragment of the B form containing residues 25–41 and 96–80, clearly indicating that those

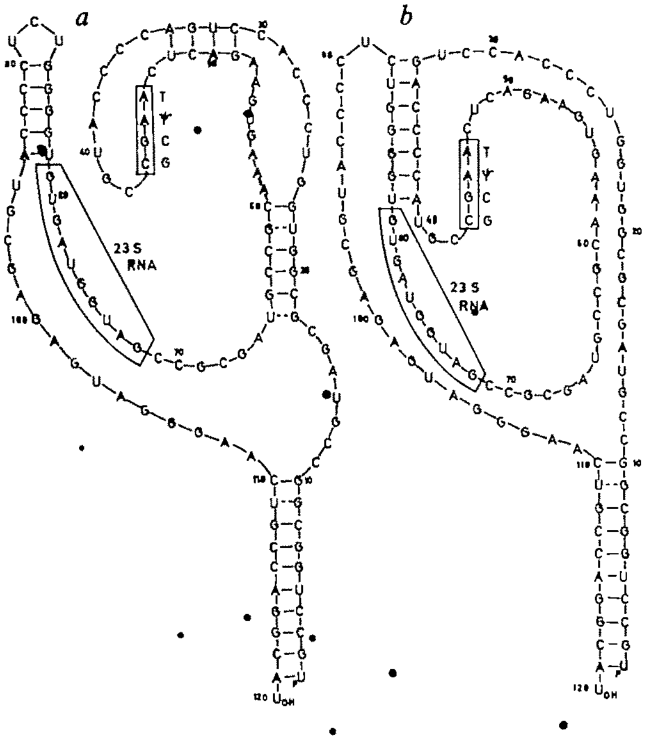
Table 1 The neighbourhood of the central helix in prokaryotic 5S RNA (frames)

E.coli[6]	28 30 ...UCCCACCU	GACCCCA	40 UGC CGAA	.....	78 80 GGU AGUG	UGGGGUC	90 UCCCCAUG...
Photobacterium 8265[3]	30 ...ACCCACCU	GAUCCC	40 UUGC CGAA	.....	80 CGU AGUGU	GGGGUU	80 UCCCCAUG...
Pseudomonas fluorescens[7]	30 ...AA CACCU	40 GAUCCCA	UCC CGAA	.....	80 GGU AGUG	UGGGGUU	90 UCCCCAUG...
Clostridium pasteurianum[8]	23 30 ...UAACACUC	CUUCCC	40 AUUC CGAA	.....	73 80 GGUACUGCAG	GGGAAG	90 CCCUGUG...
Bacillus megaterium[9]	30 ...UCACACCC	GUUCC	40 CAUAC CGAA	.....	73 GGU AGUU	80 GGGAC	90 UUUGUCCCUG...
B. stearothermophilus[10]	30 ...AAACACCC	GUUCC	40 CAUCC CGAA	.....	73 GGU AGUU	80 GGGGC	90 CAGCGCCCCUG...
B. subtilis[10]	30 ...UCACACCC	GUUCCC	40 AUAC CGAA	.....	73 GGU AGUC	80 GGGGGU	90 CUUCCCCCUG...
B. licheniformis[11]	30 ...UCACACCC	GUUCUCA	40 UGC CGAA	.....	73 GGU AGU	80 UGGGGGC	90 UUCCCCCUG...
A. nidulans[12]	24 30 ...AACCACUCUG	ACCC	40 CAUCC CGAA	.....	78 80 GAUAGCUCCC	GGGU	90 AGCCGAUCG...

sections are bonded together. This feature is found in our B-form model but not in the A-form model. The only double helices included in Fig. 1 are those which can be justified by comparative sequence analysis. The 'tuned helix', though sterically feasible in the B form, is not included because chemical modification results[5] indicate that G_{61} is accessible for modification in the B form. The high activation energy necessary for the conversion between the two forms[14] corresponds well to the melting of the specific helices.

We feel that most of the remaining structural organisation of both 5S RNA forms is probably not provided by Watson–Crick base pairing, but rather by tertiary interactions of an unknown nature. There is, however, no definitive method for distinguishing secondary and tertiary structures in RNA molecules. NMR measurement of protons in base pairs is a promising technique, but is complicated by the contribution of resonances from protons in tertiary bonds with chemical shifts similar to protons in standard base pairs[15–17]. Hence we can discount detailed models of 5S RNA structure based on NMR measurements which ignore possible contributions from tertiary bonds[18]. Our studies of conformational equilibria and dynamics in 5S RNA (in preparation) provide circumstantial evidence favouring extensive tertiary structure in 5S RNA.

Fig. 1 *a*, A-form snd *b*, B-form conformations of prokaryotic 5S RNA. Sequences complementing 23S RNA and tRNA segments are indicated.



The detailed mechanism of the 5S RNA conformational switch, and the role of GTP hydrolysis and ribosomal proteins[19,20], are subjects for potentially endless speculation. We limit ourselves to one observation. Figure 1 shows the highly conserved region G_{71}–G_{83}, which has recently been found to be complementary to a 23S RNA sequence[21]; also nucleotides CGAA in position 43–46 have been proposed[22,23] as a tRNA binding site on the basis of their complementarity with the tRNA sequence TΨCG. Note that the first base of the central helix precedes this CGAA sequence by exactly 10 nucleotides in all species but the blue-green alga *Aspergillus nidulans*, where both helix and distance are shorter by one nucleotide. The A $\rightleftharpoons$ B conformational switch, as represented in Fig. 1, places the tRNA binding site adjacent to the 23S RNA in the B form, but free to move away from that position in the A form. A switch from one conformation to another, possibly induced by the binding and release of the elongation factor G, could cause movement of the tRNA relative to the 23S RNA. As the migration of the mRNA in the ribosome is probably induced by the tRNA (ref. 24), a process of this sort is likely to be part of the translocation step in protein biosynthesis.

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matters arising

Assortative mating

VETTA[1] claims that Fisher's[2] well known formulae for the correlations between relatives under assortative mating are incorrect, and he presents alternative formulae for the parent–child and sib–sib correlations. I intend to publish elsewhere a simplified proof of Fisher's results, but it seems worthwhile to indicate here why I am not convinced by Vetta's argument.

Consider for simplicity the case in which there is no dominance and no environmental variability, so that all the variance is additive genetic. The alternative formulae for the parent–child correlation, which is in this case the same as the sib–sib correlation, are as follows

$$\text{Fisher: } \tfrac{1}{2}(1+\mu)\text{, and}$$
$$\text{Vetta: } \tfrac{1}{2}[1+\mu(1-\mu)^2]$$

where μ is the correlation between husband and wife. There is nothing in Fisher's model which restricts attention to positive, as opposed to negative, assortative mating, so that μ may meaningfully take any value between −1 and +1. Fisher's correlation increases linearly from 0 to 1 as μ increases from −1 to +1, which seems reasonable. Vetta's correlation is negative when $\mu < -0.47$, which seems implausible, and is less than −1 when $\mu < -0.87$, which is impossible. Even if we confine our attention to positive values of μ the behaviour of Vetta's correlation is strange, since it increases from 0.5 to 0.574 as μ increases from 0 to $\tfrac{1}{3}$, and then decreases back to 0.5 as μ increases further from $\tfrac{1}{3}$ to 1; I find it difficult to understand how the correlation can fail to be an increasing function of μ.

It is also quite easy to prove, at least to my satisfaction, that Fisher's correlation is correct in this case. Elementary genetic considerations show that the expected value for the child given full information about the complete genotypes of both parents is equal to the mid-parental value. Hence the regression of child on mid-parent is linear with unit slope, from which it follows that the parent–child correlation is $\tfrac{1}{2}(1+\mu)$.

Vetta claims that Fisher's formulae cannot be true in general because they predict that the parent–child correlation will in some circumstances exceed the sib–sib correlation; Vetta considers this to be impossible, but has given no reason to support this assertion.

I conclude that Vetta has failed to demonstrate that Fisher's formulae are incorrect, and that the alternative formulae presented without proof in his paper are almost certainly incorrect.

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VETTA REPLIES—I consider[1] that the assumptions on which Fisher[2] derived his formulae are not valid for his model and Bulmer[3] raises no objection to this. His objections concern the parent–child correlations not being a linear function of μ, the applicability of the model to disassortative mating and the excess of sib correlation over parent–child correlation. I consider these in turn.

Bulmer[3] claims that there is nothing in Fisher's model which restricts μ and that it "may meaningfully take any value between −1 and +1". Unfortunately, the constraints of Fisher's model are not well understood. In fact, A (= μ in Bulmer's example) cannot be increased to 1 without limit. For example, when $A \simeq 1$, the increase in additive variance is infinitely large. It is difficult to conceive of any trait with infinite variance. That assortative mating could increase genetic variance to infinity is beyond belief. It is more likely that there are genetic constraints which ensure that the increase in variance brought about by a given degree of assortative mating will not be too large. The notion that A can be increased at will, to 1, is unacceptable.

Is there any reason, apart from the formula $\tfrac{1}{2}c_1c_2(1+\mu)$, to assume that the parent–child correlation will increase linearly with μ? I know of none. Indeed, it is not absolutely clear how a non-linear increase, $A\tau^2/(1-A)$ in the additive variance should give rise to a linear increase of exactly $\tfrac{1}{2}A$ in the parent–child correlation. There is, therefore, no reason to believe that A can take any value between 0 and 1 and that the increase in the parent–child correlation should be exactly $\tfrac{1}{2}A$.

Are there any data which might indicate that an increase in μ will, necessarily, result in an increase in the parent–child correlation? Data on human populations are scarce but studies[4] on racehorses, which have been bred assortatively for generations, do not support this assertion.

Fisher[2] showed that assortative mating (AM) will introduce association between phases of factors of an individual and this will increase the additive variance of the population. Wright[5] asserted that AM will also introduce correlation between the additive and dominance deviations of mates. I show that this, indeed, happens in Fisher's model. Fisher, however, did not take acccount of such correlations. In deriving my formulae, I take account of these and thus, provide a synthesis of Fisher and Wright on AM. I show that AM will (1) increase the association between additive deviations of a parent and his progeny and (2) introduce association between the additive deviations of one and the dominance deviations of the other. These considerations require a correction term to Fisher's formulae. This term, when $c_1 = c_2 = 1$, will be $\tfrac{1}{2}A^2(A-2)$ for parent–child correlation. Note that when $c_2 = 1$,(2) is 0 but (1) is not 0.

In Fisher's model A has two meanings: it is the genetic component of μ and it also represents genetic correlation between phases of factors. It occurs in the former sense in Fisher's formulae and in the latter sense in my correction term. Obviously, when the correlation between phases of factors is 0, that is, the population is mating assortatively for the first time, my correction term is 0. I, therefore, assert that Fisher's formula for parent–child correlation, as given by Bulmer, is correct for a population which mates assortatively for the first time and is not correct for his model where the population is in equilibrium under a given degree of AM.

Geneticists[6,7] distinguish between assortative and disassortative mating because they have different genetical consequences. No polygenic model of disassortative mating is available. If one is constructed on the lines of Fisher's model. it will differ from the latter in three respects. First, there will be negative correlation between genotypes of an individual. Second, the genetic variance will decrease. And third, the correlation between the additive and dominance deviations of mates will be negative. Moreover, the attainment of equilibrium may present certain problems. Even if, for a given μ, all these effects were exactly the same in the two models, the correlational formulae may still not be the same. Fisher[2] restricts himself to a model which results in "an increase in variance". Later[8,9] discussing it, he uses assortative mating and homogamy by which he means "the tendency of mating like with like" interchangeably.

I considered the possibility that, for his model, sib correction could be greater than the parent–child correlation but rejected it. Briefly, the reason is that, in presence of partial dominance, it is greater than parent–child correlation for one factor as well as for a large number of factors[10]. This holds for assortative mating when the number of factors is small. There is no reason to believe that the situation is reversed when the number of factors is large. Fisher[2,9], himself, believed this to be the case for his model and I agree with him. For, in Fisher's model, where add., dev., dom. and cov. stand for additive, deviation, dominance and covariance respectively, sib covariance = cov.(add. dev., add. dev.) + cov.(add. dev., dom. dev.) + cov.(dom. dev., dom. dev.), and parent–child covariance = cov.(add. dev., add. dev.) + cov.(add. dev., dom. dev.). All covariances on the right of these equations are positive. Mendelian segregation will ensure that cov.(add. dev., add. dev.) and cov.(add. dev., dom. dev.) in the two equations are equal. It is obvious that in presence of dominance, sib covariance will be greater than the parent–child covariance. In absence of dominance they will be equal. Incidentally, I do not assert that it is 'impossible' for parent–child correlation to exceed the sib correlation, only that, in Fisher's model, it should not.

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Cholecystokinin produces bait shyness in rats

BECAUSE an injection of cholecystokinin (CCK) (or of the synthetic C-terminal octapeptide of CCK[1]) reduces food intake in the fasted rat, it has been suggested by Gibbs, Smith and colleagues[2–6] that CCK may be a satiety signal. We present here evidence against this conclusion.

To quote from ref 4, "In order to establish CCK as a physiological satiety signal, one requirement is that CCK does not inhibit feeding by inducing nausea or other subclinical sickness". An attempt to detect such sickness was made by injecting CCK after the rats had drunk a saccharin solution. If sickness occurs after CCK then less of the saccharin solution should be drunk on a subsequent occasion[7,8].

The tests for nausea already performed[2,3], however, were quite insensitive. In the first[2] the rats were extremely thirsty (23½ h) and were given a very attractive sweet taste to drink (0.25% saccharin). Such test conditions would only be expected to reveal a very strong nausea induced by CCK. The dose of CCK used in this nausea test suppressed eating by only 50% when the CCK was injected before the rat ate[2]. This suggests that only a mild degree of nausea was induced by that dose of CCK if nausea was indeed the explanation. The results of the test showed no effect of CCK (40 U kg^{-1}) or synthetic terminal octapeptide of CCK (40 U kg^{-1}) but a strong effect of 0.15 M lithium chloride as 0.6% body weight. Lithium chloride in this dosage is a very powerful aversive agent. Even higher levels of thirst were used in a comparison of apomorphine, another strong aversive agent, with CCK (40 U kg^{-1}) (ref. 4). Conditioned aversion was shown to apomorphine but not to CCK. Whilst these studies show that in the doses used, CCK (or the C-terminal octapeptide) is less aversive than LiCl or apomorphine, more sensitive tests of nausea might reveal intermediate degrees of aversiveness.

In our first experiment we used rats (Charles River, male Sprague–Dawley) 22-h thirsty and given 8-min exposure to a novel neutral flavour (almond Schilling 0.5% in water). Immediately after this drinking period nine rats were injected intraperitoneally with 40 Ivy dog units per kg of the terminal octapeptide in saline[6], and 8 h later the rats were given water *ad libitum* for 2 d. Then they were again water deprived for 22 h. Both groups were then again presented with the almond solution for 8 min. The mean amount consumed on the first day of exposure was almost identical for the experimental rats (10.3 ml) and for the control rats (10.4 ml), but on the second presentation the control rats drank a mean of 14 ml as against 9.6 ml consumed by experimental animals ($t=3.66$, d.f.$=16$, $P<0.01$). We used an even more sensitive indicator of conditioned aversion in our second experiment by using a preference instead of a single bottle method as a test. A group of 14 naive Sprague–Dawley rats underwent 22 h water deprivation; half the rats were then presented with almond flavoured water and the others with banana flavoured water (both 0.5% Schilling). After 24 h of water *ad libitum* the rats were again deprived of water for 22 h and allowed to drink the alternate flavour. Immediately following the consumption of this flavour, the experimental rats were injected with CCK octapeptide as above, while the control rats were injected with vehicle. After 24 h of water *ad libitum*, rats were again fluid deprived for 22 h. They were then given a 30-min simultaneous choice between the two flavoured solutions. The controls drank a mean of 8.9 ml of the flavour presented on the first day, and a mean of 8.6 ml of the fluid presented on the second day when these fluids were simultaneously presented. In contrast, the rats injected with the CCK C terminal octapeptide drank a mean of 9.4 ml of the flavour that had been presented on the first day, but only a mean of 3.3 ml of the flavour paired with the octapeptide when the two flavours were presented simultaneously. There was a significant difference between the experimental and control groups ($t=4.36$, d.f.$=12$, $P<0.001$), indicating a larger conditioned aversion due to the octapeptide measured by this more sensitive method. We therefore conclude that the C terminal octapeptide of CCK in the dose administered to produce apparent satiation causes some form of sickness in the rat. Whether CCK is a satiation hormone is therefore left in considerable doubt.

We thank Dr A. Ondetti, Squibb Institute for Medical Research, for CCK octapeptide.

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GIBBS AND SMITH REPLY—The production of a conditioned taste aversion, using a single dose of the synthetic octapeptide of CCK as the unconditioned stimulus, is not a critical test of the hypothesis that CCK mediates all or part of the satiety effect of food in the intestine. One indirect test of the hypothesis using the taste aversion paradigm would be the comparison of dose–response curves for both satiety and taste aversion If the doses of CCK required for satiety were always equal to or larger than the doses that are aversive enough to produce a conditioned taste aversion, this would be indirect evidence against the hypothesis. Such a comparison has not been performed. The hypothesis will be most critical tested, however, when the concentration of circulating, endogenous CCK can be measured reliably during satiety. Then it should be possible to decide if ingested food releases sufficient CCK to account for the satiating effect of that ingested food.

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Landscape legacy for Ireland

R. Common

The Irish Landscape. By F. Mitchell. Pp.240+32 plates. (Collins: London, 1976.) £5.50.

As an environmental scientist, and as a countryman at heart, Professor Mitchell is concerned in this book with the interactions of natural phenomena and the relationships between man and environment that are now reflected in the Irish landscape.

The author first provides an outline of the geological evolution of Ireland, emphasising the broad relationships between rock type, rock structure and denudation process in the disposition of the major relief features Professor Mitchell embellishes his narrative, from the outset, with comments that are definitive or with opinions that are open ended. He uses the map appropriately, to show space relationships, and the diagram effectively, to indicate the time sequences of formative events.

Explanatory descriptions of selected features that are associated with near-glacial and glacial conditions introduce the next chapter, which is concerned with palaeogeographical conditions in the Quaternary era. The author provides an interpretation which enlightens rather than confuses, and resists the temptation to depart from valuable generalisation for the complexities of local detail. His subsequent treatment of the development of Irish soils, plant covers and animal populations as climatic conditions, land and sea distributions changed in post-glacial time is equally thoughtful and disciplined.

Chapter 4 deals with the first farmers and their growing influence on the physical environment over a timespan of almost 4,000 yr. The findings of the archaeologist and the palaeobotanist provide the basis for the author's suggested sequence of landscape changes Amendments, no doubt, will follow as more evidence becomes available, but these should not diminish the importance of this particular contribution or the diligent research work of fellow scholars

In dealing with the landscape from early mediaeval times to 1900 the author reinterprets historical events in terms of human activities on the land. The human errors and pressures which encouraged environmental deterioration in this period seem to have influenced his thoughts on present conditions. Chapter 6, in fact, begins with a review of significant features in the contemporary scene and then considers the options for change which can enrich or degrade them. Understandably, therefore, the final chapter develops this important theme of a landscape legacy which future generations of Irish people will inherit.

This well illustrated book is written with a direct style, a clarity of thought and a turn of phrase which make for pleasurable reading. It will appeal to those with general interests in Natural History besides satisfying those with particular interests in Ireland. In academic terms it claims a significant place beside the writings of R. L. Praeger, J. K. Charlesworth and E. E. Evans. ☐

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Pheromone communication

Animal Communications by Pheromones. By H. H. Shorey. Pp viii+167 (Academic: New York and London, 1976) $16.50; £10.05.

PHEROMONES are chemicals produced by an individual which elicit specific behavioural or developmental reactions when perceived by other individuals of the same species. The presence of pheromones has been deduced in most animals, from Protozoa to primates, and in some groups, such as the insects and mammals, many pheromones have been identified chemically, and the synthetic compounds shown to have the same effects as the natural substances.

A comprehensive survey of the functions of pheromones requires a formidable knowledge of the morphology, physiology, behaviour, and even ecology, of members of almost every animal phylum The author of *Animal Communication by Pheromones* points out that no single-authored monograph on pheromones has hitherto been published, and that his book is an attempt to review, digest and present in a cohesive manner pheromone communication within the entire animal kingdom. Unfortunately, the book falls far short of these objectives.

Only brief mention is made of 'primer' pheromones, a surprising omission, since the ways in which these substances induce developmental changes in the recipients can usefully be compared in animals as diverse as the insects and the mammals, and discussion of those pheromones which induce both behavioural and developmental changes must necessarily also be limited. The arrangement of chapters dealing with particular 'classes' of pheromones—recognition, aggregation, dispersion, and so on, inevitably leads to repetition. The author's style is turgid and often leads to confusion. For example, what is one to make of: "Colonization begins with the alightment on a suitable host tree of an initially invading beetle, which may be a male or female (*but not both*), depending on the species" (p56) (my italics).

Many of the line drawings are unnecessary, and most of the plates are too dark and without contrast. Figure 14 (courtesy of N. W. Nowell) has a hopelessly inadequate legend, and must be meaningless to all but Dr Nowell, even with reference to the text And why did the publishers decide to begin each chapter on an odd-numbered page? This may be useful in multi-authored compendia, where each contributor is normally given a number of reprints, but in the present volume it results in some chapters ending with up to one and three quarters blank pages. Since £10.05 buys only 121 numbered text pages, these blank spaces are expensive. The book contains an extensive bibliography of 726 titles, together with taxonomic and subject indexes. It cannot be recommended.

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Plants and pathogens

Plant Pathosystems. R A. Robinson. Pp. x+184 (Springer: Berlin, Heidelberg and New York, 1976.) DM48; \$19 70

MR ROBINSON is a plant pathologist who has been much involved over many years in breeding crop plants for resistance to important pathogens in a number of developing countries. It is therefore not surprising when he states in his preface that this book was written in remote places far from good library facilities. This being so, one asks what would have been the difference had this been otherwise, because *Plant Pathosystems* is already a very good book and unique in many ways. The text is based on long and varied experience of field plant pathology in Africa and essentially is a series of perceptive analyses of interactions between populations of host plants and populations of pathogens, and of assessments of what such analyses teach about the management of crops so as to decrease losses caused by plant pathogens to economically low levels and, in agricultural terms, more or less permanently.

The book begins with a short account of the systems concept and of the properties, analysis and management of systems. This is useful and interesting to the uninitiated such as the reviewer. The main substance of the book starts with chapter 2, which first describes plant pathosystems, defined as any subsystem of an ecosystem which involves parasitism. It then characterises the "esodemic" and the "exodemic" as parts of epidemics that depend, respectively, on infection from within (auto-), and from outside (allo-) crops and which are therefore of quite different significance in the development of epidemics especially in relation to the type of disease resistance that controls infection.

Chapters 3–6, as the core of the book, deal in depth with analysis and management of pathosystems comprising either interactions between vertical resistance and vertical pathogenicity, or between horizontal resistance and horizontal pathogenicity. Subjects discussed include the properties of vertical and horizontal resistance in genetical and epidemiological terms, the nature and role in disease of gene-for-gene relationships and of strong genes, genetic flexibility of host and pathogen in relation to the origin and developments of epidemics, and the paramount importance of horizontal resistance in crop management both generally and as illustrated by many well-chosen examples of important diseases of crops of different types, and especially of maize and sugar-cane.

Chapter 7 is concerned with the operation of some of these factors in polyphyletic crops, again illustrated with examples, and chapter 8 is a short review of the nature and assessment of the vulnerability of crops to serious damage by pathogens, and how vulnerability can be decreased by various measures which prevent, or prepare for, the exposure of crops to virulent pathogens.

Chapter 8 contains a series of conclusions which emphasise the value of the systems concept and of well conceived programs of domestication of crop plants which exploit the superior qualities of horizontal resistance. A last chapter on terminology defines or describes, often at some length, no fewer than about 300 words, terms and concepts in some 24 pages. This is a particularly valuable and essential part of the book. There are about 100 references, a small number in relation to the scope of the book; even so, a few are unexpected. The shortness of the list is explained by the circumstances and style in which the book was written. In contrast, there is an extensive index of about 1,000 entries.

Mr Robinson states in his Preface that his book is theoretical and speculative. But much of it is a good deal more than theoretical, as would be expected from Mr Robinson's background; and the speculation is reasoned and not unrealistic. Quite a lot of the book is controversial. Thus, Mr Robinson's concept of vertical and horizontal resistance will be regarded as oversimplified by plant pathologists who have studied resistance at the cellular and molecular levels. Also, Mr Robinson does not like the extensive use of vertical resistance in plant breeding and says so very plainly. But he is an enthusiast for the use of horizontal resistance whenever possible and that, in his opinion, should be almost always.

Some plant breeders will not agree with the extremities of his approach. The writing from time to time is forthright and provocative but attractively so and certainly should not offend those against which it is sometimes quite pointedly directed. The book is well and clearly written but must be taken slowly because almost each paragraph on each page contains one or more substantial points which must be understood if the main themes which are propounded are to have their full impact. But the effort called for is very well rewarded because few books published on plant pathology during the past three decades are so stimulating, challenging, and have been written with such worthy objectives. *Plant Pathosystems* will undoubtedly cause quite a stir among plant pathologists and plant breeders, for most of whom it must be regarded as compulsory reading.

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Laser physics

Principles of Lasers. By O. Svelto. Pp. x+376 (Plenum: New York and London, 1976.) \$30.00.

THERE is light, and light. Or as Svelto might put it, the properties and quality of radiation can be defined and quantified by the first, and higher-order, correlation functions of the electromagnetic field. Thus, to first-order the coherence of laser light is revealed in its exceptional brightness and monochromaticity. The unique spatial and temporal coherence of such sources has already led to a number of practical applications, and largely accounts for the current scientific interest in this subject.

This book develops the theory and techniques of laser physics from first principles (quantum mechanics and electromagnetism), is completely self-contained, and should prove of particular value to the undergraduate reader. The text is well illustrated, very carefully referenced, and includes imaginatively chosen questions at the close of each of its nine chapters. (Problems in the introductory chapters, for example, range from black-body radiation to practical design problems of ruby, dye, He-Ne and CO_2 lasers.) Pumping processes, passive optical resonators, continuous and transient energy extraction (including the semiclassical treatment of π pulses, photon echoes and passive-mode-locking) are treated in detail; illustrative applications, including holography and optical data-processing, are treated more generally. Topics involving quantisation of the electromagnetic field (such as laser noise) are not discussed.

In my opinion, unstable optical resonators warranted a more extensive discussion for example, the importance of diffuse apertures was not considered, and Ananev's work was not referenced. Surprisingly, the highly efficient CO laser and recent Kr_2^* and Ar_2^* excimer (and halide exciplex) lasers receive no mention. These are, however, minor criticisms of a book which has been carefully translated and which makes excellent reading.

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WILEY INTERSCIENCE

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edited by **A. H. Riesen,** *University of California, Riverside,* and **R. F. Thompson,** *University of California, Irvine.*

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Circle No. 08 on Reader Enquiry Form.

Practical Methods in Electron Microscopy, Volume 5

edited by **AUDREY M. GLAUERT,** Strangeways Research Laboratory, Cambridge, England.

1977. about 556 pages. Price: US $71.25/Dfl. 178.00. ISBN 0-7204-0605-6. Hardbound.

The books in this series provide enough detailed information for newcomers to carry out well-established techniques with success. Each volume is published in a hardbound edition for reference purposes and individual parts are published in paperback for easy use in the laboratory.

Part I

Staining Methods for Sectioned Material

by **P.R. LEWIS,** Physiological Laboratory, University of Cambridge, England, and **D.P. KNIGHT,** Biology Department, King Alfred's College, Winchester, England.

1977. about 332 pages. US $22.50/Dfl. 56.00. ISBN 0-7204-0606-4. Paperback.

The aims of this part are twofold: to help the beginner to choose between the bewildering variety of published techniques and to provide a compact source-book of those particular techniques which have proved to be both useful and reasonably easy to use. To achieve the first aim some discussion is included of the underlying chemical basis of the staining techniques. Acquaintance with histochemical techniques at the light microscopical level will help in the understanding of the more complex staining procedures described but it is anticipated that the beginner will find this volume self-sufficient. Some of the more complex cytochemical techniques are included, but the main emphasis is on providing full practical details of basic and dependable methods for use by the average electron microscopist, rather than on covering all the latest modifications and applications.

Part II

X-ray Microanalysis in the Electron Microscope

by **JOHN A. CHANDLER,** Tenovus Institute for Cancer Research, Welsh National School of Medicine, Heath, Cardiff, Wales.

1977. about 232 pages. Price: US $17.75/Dfl. 44.00. ISBN 0-7204-0607-2. Paperback.

This laboratory manual describes, for the uninitiated and experienced alike, the principles of X-ray microanalysis together with the range of instruments and techniques available for producing, measuring and interpreting the X-ray signals. The preparation and handling of thin specimens in the TEM and thicker samples in the SEM are discussed in detail for both biological and physical materials. Throughout, major emphasis is placed on practical details, and a wide range of applications is used to illustrate the practical problems involved in specimen preparation and analysis.

Clearly illustrated to provide an easy-to-read and comprehensive reference to all aspects of X-ray microanalysis in the electron microscope, this book should find a place in laboratories of cell biology, zoology, chemistry, mineralogy, physics and metallurgy.

In preparation

Volume 6 Part I Autoradiography and Immunocytochemistry

Part II Quantitative Methods in Biology

by **M.A. WILLIAMS,** Reader in Human Biology and Anatomy, University of Sheffield, England.

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Circle No. 09 on Reader Enquiry Form.

Calcium in control

Calcium in Biological Systems. (Symposia of the Society for Experimental Biology.) Edited by C. J Duncan. Pp. viii+485. (Cambridge University: Cambridge, London and New York, 1976.) £18.

THE study of extracellular calcium metabolism and its hormonal control has progressed steadily over many years. Interest in intra-cellular roles of calcium, confined for some time to its regulatory function in muscle, has recently grown rapidly following independent advances in several areas. Calcium is now known to be a key factor in triggering secretion, activating cells for division, controlling intercellular communication and regulating glycolysis and respiration, in addition to its involvement in contractile systems and in nervous conduction.

This latest Symposium of the Society for Experimental Biology considers mainly these intracellular systems. The organisers have concentrated attention on the general problems of how intracellular calcium levels are controlled, rather than on the detailed analysis of the effector roles of Ca^{2+} which differ greatly in different systems. This is a particularly difficult experimental field for the very reason that calcium is such a good controlling ion; its intracellular concentration is normally in the submicromolar range so that small fluxes have large effects.

It is only within the last five or ten years that the discovery of aequorin by Shimomura and Johnson has provided a direct approach to its measurement. These authors contribute a useful paper on the properties of this interesting luminescent protein and other papers by P. F. Baker and C. C. Ashley *et al* describe its application in nerve axons and in muscle fibres. Baker also provides a good general analysis of intracellular Ca^{2+} regulation, and Ashley *et al.* consider the technical problems in the quantitative interpretation of aequorin luminescence They also describe the use of a microscintillator probe to follow the influx of $^{45}Ca^{2+}$ but they do not evaluate the relative merits of these two techniques.

Two good introductory chapters deal with the general chemistry of Ca^{2+} and its complexes (R. J. P. Williams) and with a variety of natural and synthetic ionophores for divalent cations (M. Truter). I missed a similar introductory section on the use of electrophysiological techniques and concepts and the uninitiated will find unfamiliar jargon and usage in this field. For example, the significance of a potentially interesting paper on the effects of intracellular calcium on membrane permeability (R. W. Meech) was obscured by the use of the term 'activation' in different and undefined senses.

Almost half of the twenty papers concern contractile systems and three of these were on troponin. A clear account of its evolutionary relationships with parvalbumins and myosin light chains, based on amino acid sequences (J. H. Collins), was complemented by a comparison of control functions in different species and a study of the functional interchangeability of these regulatory proteins *in vitro* (A Szent-Gyorgyi). It was pointed out (S. Ebashi *et al.*) that in spite of such homologies one must be cautious about arguing too closely from the regulatory mechanisms in skeletal muscle to other situations, since in smooth muscle, for example, calcium behaves as an activator rather than as a derepressor.

Several papers considered the problems of coupling between excitation and contraction. In skeletal muscle (S. R. Taylor and E. Godt) a depolarisation induced release from sarcoplasmic reticulum is the main physiological mechanism. The more complex situation in heart muscle is reviewed at the physiological level by R. Niedergerke *et al*, and some of the biochemical problems are discussed by E. Carafoli and M. Crompton in their critical evaluation of the role of mitochondria in Ca^{2+} regulation.

One conclusion to be drawn from several of the contributions is that with the exception of sarcoplasmic reticulum and of Ca^{2+} uptake by mitochondria, little is known about the nature of intracellular calcium stores and their control. The large reservoir of calcium in the nucleus can best be described as a pool of ignorance.

The conference is ably summarised in a concluding chapter (A. Weber) which also mentions relevant points from the discussion These tantalising glimpses make one wish that a more extensive record of this part of the meeting could have been included in the book. Nevertheless, the account as it stands provides a valuable survey of a diversifying and developing subject. It will be particularly valuable to workers in the calcium field who wish to keep up-to-date with advances in less familiar parts of their subject.

N. Michael Green

N. Michael Green is a member of the MRC staff, working at the National Institute for Medical Research, London, UK.

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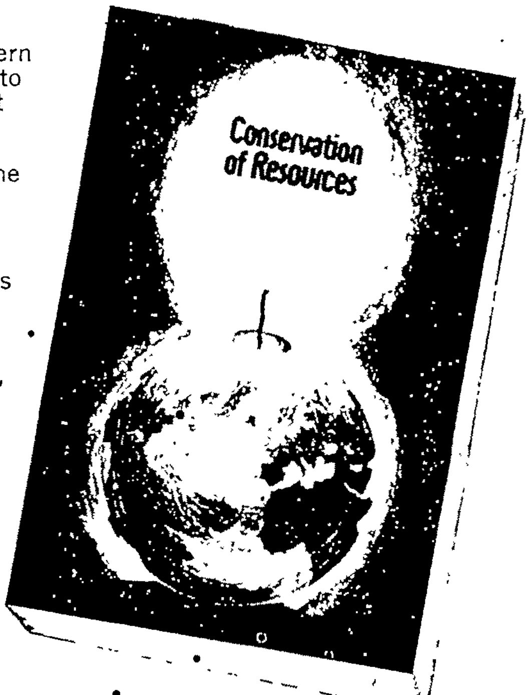

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Circle No. 12 on Reader Enquiry Form.

Pragmatics

Language and Context: The Acquisition of Pragmatics. (Language, Thought and Culture: Advances in the Study of Cognition.) By E. Bates. Pp. xiv+375 (Academic. New York and London, 1976.) $19.95; £12 20.

PSYCHOLINGUISTICS has traditionally depended on linguistics proper to supply research topics. When linguists produce a rule to which the sentences of our native language can conform, psycholinguists can then go off and try to explain either the sense in which we "know" this rule, or how we manage to act as if we knew it. Until quite recently, the rules in question were almost exclusively rules of syntax, and the psycholinguist's problem was, for instance, to explain the mental organisation that might lead us to exclaim "Ah, there's the book that I was hunting for!" but never "Ah, there's the book that I was wondering where was!".

In the past few years, however, linguists have been giving increasing attention to the study, under the heading of "pragmatics", of the conventions surrounding the use of various linguistic forms. For instance, "Can you shut the window" has a syntactic form normally associated with questions, yet it might easily function as a request. Furthermore, it is a more polite request than a simple "Shut the window". In some circumstances, "Do you think it's a bit chilly in here?" might be more polite still, although at other times it will just be annoyingly indirect.

The psycholinguist Elizabeth Bates has set out to chart the mastery by children of these, and other, subtleties of linguistic usage. Working from studies of pre-school age Italian children, she marks the onset of such phenomena as talking about talking, laying emphasis on particular words, discussing situations beyond the here and now, and wording an appeal for a sweet so as to make it as persuasive as possible She also considers, from the standpoint of Piaget's theory of cognitive development, what mental capacities are required to reach these pragmatic milestones, and so sets linguistic development in the context of general cognitive development.

Professor Bates has written an excellent and fascinating book. She is considerate to the reader, explaining clearly and carefully both the theoretical background, and the design and interpretation of her experimental studies It is a book pregnant with possibilities for further research, and one can only hope that others who follow her, mantain her standards of liveliness and clarity. **S. D. Isard**

S. D Isard is Lecturer in Experimental Psychology at the University of Sussex, UK.

Air pollution

Effects of Air Pollutants on Plants. (Society for Experimental Biology, Seminar Series 1.) Edited by T A Mansfield Pp. 209. (Cambridge University. Cambridge, London and New York, September 1976.) Hardcover £8 50; paperback £3 80.

THIS volume appears 15 months after the seminar on which it is based. The authors have concentrated on their own findings, and consequently much material appears here for the first time; coverage is uneven and references highly selective. Less understandably, there is no attempt to place the contributions in a wider framework or to define the outstanding problems. For example, the chapter on uptake of pollutants (Unsworth, Biscoe and Black) mentions only the resistance analogy approach; such work surely should form the basis for simulation modelling of the growth of polluted crops. but nowhere in the book is this suggested. Only Bradshaw on evolution and pollution gives an integrated account of his field The central challenge of bridging laboratory and field observations is raised both for fluorine (Davison and Blakemore) and for ozone (Manning and Feder)—the latter authors, like most Americans, eschew growth analysis. Both of these chapters find contradictions between laboratory and field data: could the method of expressing pollutant levels (as mean, peaks or dose) be partly responsible? The singular properties of open top chambers (McCune, Maclean and Schneider) make it unlikely that this type of enclosure will yield unambiguous results By recording changes in enzyme activities following fumigations, the Lancaster group point the way to pollutant-specific physiological tests Would that more work on SO^2 and lichens were as analytical as that described here (Nieboer, Richardson, Puckett and Tomassini) Two useful appendices summarise data on air chemistry and references to biochemical effects. Overall, we are given valuable data but little perspective; a book for the specialist

J. F. Farrar

J F. Farrar is Lecturer in the Department of Botany at the University of Liverpool, UK.

Soils of arid regions

Soils of the Arid Regions. (Developments in Soil Science 6.) By H. E. Dregne. Pp. xii+237. (Elsevier Scientific. Amsterdam, Oxford and New York, 1976.) Dfl. 75.00; $28.95.

THE aim of this book in attempting to collect together the available information on arid region soils is highly commendable. In attempting to cover such a vast topic in only 223 pages, however, the author sets himself an impossible task. The inevitable result is a book which is too specialised for the layman and too superficial for the professional pedologist.

From a brief introduction to arid regions (excluding polar deserts) the author moves on to consider with little comment, various approaches to the classification of arid region soils, finally selecting the USDA 7A scheme as a basis from which to work. Chapter three consists of a short discussion of each of the soil orders found in arid regions together with a cursory summary of the geomorphology of arid regions. In the following six chapters the distribution of arid soils is considered on a continental basis; accompanying each of these chapters is an absurdly small scale map showing the distribution of the principal associations of great soil groups. The choice of symbols is such that on several maps it is impossible to differentiate between alfisol, aridisol and mollisol groupings. This approach to the consideration of soil distribution leads to a mass of needlessly repetitive information on each great group, which could, together with better maps, have been condensed into one chapter. The last three chapters cover very briefly those chemical physical and biological properties of soils which are (according to the author) important for management. Throughout the whole text discussion on pedogenesis is extremely limited and very superficial.

The standard of the twenty-six line illustrations (there are no photographs) is extremely low: several are illegible and many others are irrelevant, adding nothing to the text.

Despite all these shortcomings, had the book been reasonably priced it could have been recommended, simply because it is the first text to attempt to draw together information on arid region soils With its outrageous price of about £19.00, brief coverage and appalling illustrations, however, there is no reason to recommend this book. Sadly there still remains a great need for a comprehensive text on the soils of arid regions. **M. J. Alexander**

M J. Alexander is Lecturer in Geography at the University of Durham, UK.

obituary

Professor Philip Macdonald Sheppard, FRS, died on October 17, 1976. He was an outstanding scientist, an inspiring teacher, a formidable adversary and a very good friend.

Philip Sheppard was born in 1921, the son of a schoolmaster. He was educated at Marlborough College. He joined the Royal Air Force Volunteer Reserve in 1940, and was a prisoner-of-war from 1942 to 1945. Immediately after the war he went up to Worcester College, Oxford, to read Zoology, and in 1948 was awarded a second class Honours Degree. It is said that he could easily have gained a 'first' had it not been for his single-minded devotion to genetics, the subject to which he dedicated the rest of his life.

He studied for the D.Phil. at Oxford under Dr (now Professor) E. B. Ford, and obtained the degree in 1951. From 1951 to 1956 he was Junior Research Officer in the Department of Zoology, taking a year off in 1954 to work with Professor Theodosius Dobzhansky at Columbia University. In 1956 he was appointed Senior Lecturer in Genetics in the Department of Zoology at Liverpool University. He was promoted to Reader in 1959. In 1963 he became the first Professor of Genetics at Liverpool, and remained in this post until his death. He was elected to a Fellowship of the Royal Society in 1965, and to an Honorary Fellowship of the Royal College of Physicians in 1975. In 1974 he was awarded the Darwin Medal of the Royal Society, and in 1975 the Gold Medal of the Linnean Society.

The bare description of a distinguished career shows little of the great impact that Philip Sheppard made on the study of ecological genetics. He had an almost uncanny ability to see the next step in an argument, the next experiment to be done. He applied this ability freely and generously to the work of others, and generated many an idea or experiment that came to be published under another name

His own work completed the transformation of ecological genetics from a science of observation to a science of experiment. His central interest lay in the mechanics of natural selection and its effects on the genetic constitution of organisms. This interest guided his work with Sir Ronald Fisher and Professor E. B. Ford on polymorphism in the moth *Panaxia dominula*, in which they finally demonstrated that the temporal changes of gene frequency were the consequences of natural selection. It motivated his studies with Professor A. J. Cain on polymorphism in the snail *Cepaea nemoralis*, studies which showed that spatial variations in this species were also due to selection, and which culminated in his beautiful experiments on selective predation by thrushes. It also guided the work with Sir Cyril Clarke on mimicry in butterflies of the genus *Papilio*, demonstrating the evolution of 'supergenes', linked polymorphic complexes of genes maintained in a state of linkage disequilibrium by strong selective interactions. Their interest in such interactions led Sheppard and Clarke to studies of human blood groups, bringing them finally to a method of preventing Rhesus haemolytic disease. Apart from these classic experiments, Sheppard also made important studies on the evolution of dominance, the genetics of industrial melanism in moths, heavy metal tolerance in plants, insecticide resistance in mosquitoes, and schizophrenia, anencephaly and spina bifida in man. In 1958 he published *Natural Selection and Heredity*, and the fourth edition was issued shortly before his death It is still the best introductory book about ecological genetics, and one of the rare breed of elementary texts that are quoted in the research literature because of the original ideas that they contain.

He was an exceptional teacher. His transparent intellectual honesty, his passion for truth and rigour, and perhaps above all his patent desire that his hearers should understand and enjoy his subject, made an enduring impression on his students. As a colleague and friend he was a delight, and a very good companion. His comments on cherished ideas or manuscripts were sometimes blunt to the point of pain, but they were always given with good humour, never tinged with malice. His friends said that he reversed the normal academic procedure, he stabbed you in the front, and then did you favours by stealth Hating pomposity, he was marvellously uncorrupted by success. As a distinguished Darwin Medallist he was much the same Philip Sheppard as the Junior Research Officer at Oxford. He bore his three-year illness with quite extraordinary courage and cheerfulness.

His life can be epitomized in the words of one who was also a prisoner-of-war. "To serve his vision, to protect it against all plausible substitutes, reasonable approximations and coward compromises is still, I believe, the knightly duty of contemporary man. He has only to remain steadfast in pursuit of it and his life will achieve something which is greater than happiness and unhappiness: and that is meaning."

B.C.C.

Sigurd Zienau died on October 18, 1976 at the early age of 55 years. He had been a Reader in Physics at University College London since 1965, following a period from 1954 as a Lecturer, and as ICI Research Fellow at Liverpool.

Dr Zienau was trained in the old school of European physics, being a student of Heitler, Pauli and Fröhlich, after a distinguished period as a mathematics undergraduate at Birkbeck College.

His name is familiar to solid state physicists as a co-author of the theory of the polaron through which, in 1950, field theory was introduced into solid state physics. He will also be remembered in the worldwide community of theoretical physicists for his important revisions of two classic texts in theory: both Heitler's *Quantum Theory of Radiation* (3rd ed.) and Mott and Massey's *The Theory of Atomic Collisions* (3rd ed.) owe much to his labour and insight. He had a phenomenal memory for the literature of theoretical physics and would recall from obscure journals published in the first half of the century the seminal work that could shed light on modern problems. His main research interests lay in scattering theory and electrodynamics, but he had a scholarly interest in a much wider range of subjects.

Dr Zienau's published papers do not, in quantity, do justice to his gifts. However, they are thoughtful contributions and each one represents the distillation of many months hard work, as well as hard argument, with his co-workers. There are three phases of his research represented in these papers. In the early 50s he published work on dielectrics, especially on the motion of slow electrons in polar materials. Later in the 50s and early 60s he published a series of six papers on electrodynamics including contributions to damping processes, line shapes, intermolecular forces (including retardation and three body interactions) and gauge problems. The final set of papers concern various specialised aspects of field theory.

He trained about ten research students who now teach throughout the world and these will always have that special magic that comes from having worked with and learned from Sigurd.

In recent years he interested himself in the history and philosophy of science and was active in the British Society for Social Responsibility in Science.

He will be sadly missed by his friends, colleagues and students at University College. He leaves a young daughter and son by his second marriage, and one son by his first.

E. A. Power
F. F. Heymann

J. V. Peive, the Soviet soil scientist, died after a long illness on September 13, 1976, aged 70. As well as being one of the leading Soviet agricultural scientists of the post-war era, Dr Peive had been politically active and, according to Tass, his official obituary was signed by Mr Brezhnev and other prominent Soviet politicians and scientists. Of Latvian extraction, he was born in 1906 in the adjoining Toropets district of Russia and, after training as a teacher, graduated in 1929 from the Timiryazev Agricultural Academy in Moscow.

For 13 years from 1931 he worked in the All-Union Flax Research Institute in Torzhok, some 200 km north-east of Moscow, rising to the position of Director. After the war he became Rector of the Latvian Agricultural Academy in Riga, carrying out his own specialist investigations in the Laboratory of Biochemistry of Soils and Trace Elements in the Institute of Biology. He published a text-book on the biochemistry of soils in 1961 but will be best remembered for his work on the effects of trace elements in soils on plants and animals. Several volumes of the Transactions of his laboratory, dealing with different aspects of trace element problems, have been published by the Latvian Academy of Sciences, and he chaired the Co-ordinating Commission on Trace Elements in Soils, Plants, Animals and Human Nutrition in the USSR. Dr Peive's wider interests in soils were recognised by his appointment as Editor-in-chief of *Pochvovodenie*, the Soviet journal of soil science, a post that he had occupied for the past 14 years.

Peive rose to the Presidency of the Latvian Academy of Sciences and, after 13 years as a Corresponding Member, was elected an Academician of the Academy of Sciences of the USSR in 1966, in which he held the post of General Secretary. He was Prime Minister of Latvia from 1959 until 1962 and was one of the three Latvian representatives in the Soviet of Nationalities, one of the two Chambers of the Supreme Soviet of the USSR, and Chairman between 1958 and 1966.

He seldom attended scientific gatherings outside Russia, although only ill-health prevented his participation in the meeting of Commissions II and IV of the International Society of Soil Science in Aberdeen in 1966. In private discussion he talked about his work with much less formality than is the case with many Soviet scientists and often introduced a light-hearted note. His major contribution to Soviet agriculture was in the immediate post-war years when he pointed out the existence of trace element problems in many Russian soils, particularly deficiencies of boron, zinc, manganese, cobalt, copper and molybdenum.

R. L. Mitchell

Professor Leo Pincherle died suddenly on October 25, 1976 I came to know him well during the last years of his life He regularly attended the Bedford/Westfield College solid state physics seminars. Characteristically, he spoke little at these meetings, but his rare contributions were profound and to the point. He was modest to a fault and abhorred the limelight; yet his colleagues, friends and students know of the innumerable unobtrusive ways in which he served the cause of scholarship in teaching and research.

Leo Pincherle was born in Bologna in 1910. He came from a line of distinguished academics—the mathematician Salvatore Pincherle was a grandfather—but Leo was the first of the family to take up physics as a career. His first publication (1931) reported a calculation of the wavelength of the oscillations in Hull's magnetron; and the interest in the theoretical principles underlying practical devices marked his work to the end. He was with Fermi in Rome, later moved to Padua and came to England shortly before the Second World War as a refugee from Mussolini's Italy During the difficult years which followed he was befriended by the late H T Flint who brought him as a lecturer to King's College, London. Leo's early research, on a variety of topics in spectroscopy, atomic physics and electromagnetic theory, demonstrates the width of his scientific interests. But his great productive period came at the Telecommunications Research Establishment (TRE), Malvern, where he was Principal Scientific Officer from 1948 until 1955. Here he established himself as a leading authority on energy band theory. The 1953 paper on the band structure of lead sulphide (Bell *et al.*), in which the cellular method was first applied to a diatomic semiconductor, is an early classic in the field, and fundamental theoretical work on applications of group theory appeared in the form of TRE Memoranda Pincherle returned to university life in 1955 when he rejoined Flint, by then head of the physics department at Bedford College, London, as a University Reader; overdue promotion to Professor of Mathematical Physics came in 1969. A fruitful collaboration with P. M. Lee led to further band structure papers in the early 1960s. In the last ten years of his life Leo Pincherle published few research papers. But he continued to be active and fertile in the many other ways a scholar exerts his influence: as supervisor of research students, as a sought-after contributor to summer schools in Italy, as UK editor of the international journal *Solid State Electronics,* and not least as author of the definitive textbook *Electronic Energy Bands in Solids* (1971) which itself contains much original work. Above all Leo Pincherle loved teaching and the contact with his students, a love which was fully returned, and the prospect of retirement from this activity filled him with dismay. Leo's lectures and publications all bear the stamp of his characteristic approach to physics careful preparation, great clarity and economy of style, the use of the simplest mathematical techniques appropriate to the physics of the problem in hand. He tackled difficult problems but was never a narrow specialist.

The picture of Leo Pincherle the scientist cannot be separated from that of Leo Pincherle the man. He was devoted to his family, for whom his too-early death is a tragic loss. His sense of humour, dry and delicious, showed in his fund of stories about well-known contemporaries — always amusing and never malicious. His quiet demeanour concealed plenty of fire and imagination, as you soon discovered when you partnered him at bridge. He was the most knowledgeable of men on fundamental aspects of European culture and history and wore his learning lightly; his love of music and the arts was intense, his taste discriminating. He was, in the best sense, both very Italian and very English, combining in his person that internationalism in science and that European spirit which it is so necessary to keep alive today. His friends miss him deeply.

Ernst Sondheimer

announcements

Awards

The Lomonosov medals—the highest award of the Soviet Academy of Sciences—for 1976 have been awarded to **Academician S. I. Vol'fkovich** of the Soviet Academy of Sciences for his work on the chemistry of phosphorous, and to **Dr Hermann Klare**, President of the Academy of Sciences of the German Democratic Republic, for his work on artificial fibres.

The Royal Astronomical Society Gold Medal has been awarded to **Mr John Bolton**, a Chief Research Scientist with the CSIRO Division of Radiophysics at Parkes, NSW, for outstanding contributions to both radio and optical astronomy, particularly for his studies of radio sources in space.

The Clough Medal for 1976–77 has been awarded to **Professor T. S. Westoll**, FRS, Department of Geology, University of Newcastle upon Tyne for contributions to geology, in particular studies of fossil vertebrates and their application to the stratigraphy of the Old Red Sandstone of Northern Scotland. The Clough Award for 1976–77 has been conferred on **Dr E. N. K. Clarkson** of the Grant Institute of Geology, University of Edinburgh, for his work in palaeontology and palaeoecology, in particular on the eyes of trilobites and including trilobites from the Lower Palaeozoic rocks of Scotland

The Faraday Medal has been awarded to the Executive Director-General of the CERN Laboratories in Geneva, **J. B. Adams**, for his contribution to the design and construction of high-energy particle accelerators.

Meetings

14 March, **Ion Implanted Semiconductor Devices**, London (Institution of Electrical Engineers, Savoy Place, London WC2).
15 March, **Vehicle Navigation and Identification**, London (Institution of Electrical Engineers, Savoy Place, London WC2).
26–28 March, **Changing Sea Bird Populations Of the North Atlantic**, Aberdeen (G Sarle, Royal Society for the Protection of Birds, Sandy, Beds.).
29 March–1 April, **Submillimetre Waves and their Applications**, Surrey (Institute of Physics, 47 Belgrave Square, London SW1).
5–7 April, **Energy in the 80s**, Middlesbrough (Institution of Chemical Engineers, 165–171 Railway Terrace, Rugby).
18–20 April, **Advances in Extractive Metallurgy**, London (Institution of Mining and Metallurgy, 44 Portland Place, London W1).
19–20 April, **Strain Rate Method of Stress Corrosion Testing**, Newcastle upon Tyne (Society of Chemical Industry, 14 Belgrave Square, London SW1).
9–11 May, **Hepatotrophic Factors**, London (Ciba, 41 Portland Place, London W1).
17 May, **The Changing Face of Cancer**, London (Marie Curie Memorial Foundation, 124 Sloane Street, London SW1).
22–25 May, **Autoradiography in Pharmacology and Toxicology**, Uppsala (S. Ullberg, University of Uppsala Biomedical Center, Box 573, S-751 23, Uppsala, Sweden).
31 May–2 June, **Lasers in Chemistry**, London (M. West, The Royal Institution, 21 Albemarle Street, London W1).
1–2 June, **30th Chemists' Conference**, Scarborough (K. Speight, BSC, Sheffield Laboratories, Hoyle Street, Sheffield).
15–26 August, **Scientific and Mathematical Foundations of Engineering Acoustics**, Cambridge, Mass. (MIT, Room 3-366, Cambridge, Mass. 02139).
31 August–2 September, **Cell–Cell Recognition**, Oxford (A. S. G. Curtis, Cell Biology, University of Glasgow, UK).
4–8 September, **Low Energy Ion Beams**, Salford (Institute of Physics, 47 Belgrave Square, London SW1).
10 September, **The Microscopic Structure and Dynamics of Liquids**, Corsica (J Dupuy, Physics, University of Lyons, 69621 Villeurbanne, France).
13–16 September, **Scintillation Counting**, Bath (The Secretary, Analytical Division, Chemical Society, Burlington House, London W1).
26–28 September, **On-line Surveillance and Monitoring of Process Plant**, London (E. Wakely, Society of Chemical Industry, 14 Belgrave Square, London SW1).
2–4 October, **5th Biennial Symposium on Turbulence**, Rolla, Missouri (J. L. Zakin, Chemical Engineering, University of Missouri, Rolla, Missouri 65401).
10 October, **The Display of Nondestructive Testing Data**, London (Institute of Physics, 47 Belgrave Square, London SW1).
19–21 October, **Current Topics in Drug Research**, Uppsala (R. Dahlbom, Biomedical Center, University of Uppsala, Box 574, S-751 23, Uppsala, Sweden).
9–11 November, **Atherosclerosis**, Milan (Fondazione G. Lorenzini, Via Montenapoleone 23, Milano, Italy).
28 November–2 December, **Protons and Ions Involved in Fast Dynamic Phenomena, Paris** (C Troyanowsky, 10 rue Vauquelin, 75231 Paris Cedex 05, France).
23–28 February 1978, **5th International Wheat Genetics Symposium**, New Delhi (M. V. Rao, Cummings Laboratory, Indian Agricultural Research Institute, New Delhi–110012, India).
10–14 April, **9th International Symposium on Carbohydrate Chemistry**, London (J. F. Gibson, The Chemical Society, Burlington House, London W1)
12–14 April, **4th European Immunology Meeting**, Budapest (The Secretariat, c/o MOTESZ, H-1361 Budapest, Hungary).
17–20 April, **10th National Atomic and Molecular Physics Conference**, London

Person to Person

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There will be no charge or this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

(Institute of Physics, 47 Belgrave Square, London SW1).

20–25 August, **3rd European Conference on Analytical Chemistry,** Dublin (Euroanalysis III, Institute for Industrial Research and Standards, Ballymun Road, Dublin 9, Ireland).

4–7 July, **7th International Cryogenic Engineering Conference and Exhibition,** London (Institute of Physics, 47 Belgrave Square, London SW1).

20–25 August, **Plant Cell and Tissue Culture,** Calgary (IAPTC Congress, University of Calgary, Calgary, Alberta, Canada).

21–25 August, **4th International Congress of Cybernetics and Systems,** Amsterdam (J. Rose, c/o College of Technology, Blackburn, UK).

4–8 September, **4th International Symposium on Physical Organic Chemistry,** York (J. F. Gibson, The Chemical Society, Burlington House, London W1).

11–15 September, **Thin Film Properties in Relation to Structure,** Loughborough (Institute of Physics, 47 Belgrave Square, London SW1).

Reports and Publications

Other countries—January

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nature

17 March 1977

More Interdisciplinary Theses

IN the past couple of years, academics in Britain have been urged by the Science Research Council to look at the style of postgraduate education provided by the Massachusetts Institute of Technology (MIT) and to think seriously about transplanting some of its best features to British universities. Particular attention has been drawn to the extensive course work done in the first year or two at the postgraduate level, the subsequent examination, success in which has been necessary before a thesis could be ventured into seriously, and the (by British standards) free-flowing interdisciplinary character of much of the work that a student would do. But how does MIT see its own intellectual future—is there to be more innovation in postgraduate education or is the present mixture regarded as about right?

MIT administrators see a variety of trends as both restricting and demanding future change. There is a general feeling that the shape of postgraduate education in terms of requirements is satisfactory and should not be tampered with. But the content is another matter; defence and space were the two big customers for MIT PhDs and for research projects at the institute in the 1960s, and this wave has now broken. Present day and near-future demands on science and engineering research are much more closely related to what one person described as 'ways of engaging reality', such as increasing industrial productivity, solving energy problems and looking at questions in biomedical engineering and in transportation. These are interdisciplinary problems *par excellence* and there seems general agreement that the interdisciplinary revolution at MIT has not yet run its course. There is a pretty clear lesson here for some of the narrow self-regarding departments in British universities.

New commitments to multidisciplinary research and education are bound, however, to come up against the severest of financial and administrative obstacles. A report last summer by an MIT committee charged with looking at the institute's research structure painted a generally gloomy picture of federal support levels which have stagnated for several years, and of the research environment, which many believed had declined in recent years largely as a result of increasing pressure on faculty to raise research funds and a concomitant drop in flexibly-available resources. The committee endorsed the singular mix which MIT had evolved of departments, inter-departmental research programmes and specific laboratories and saw, in particular, research centres on campus as a way to proceed for the next decade; such concepts would be bound to influence the content of the education provided.

But if there are still new academic horizons, however shaky the funding prospects, there is one factor which is causing a lot of worry: changing levels of student enrolment in the next twenty years. Projections for the next ten years by the National Center for Education Statistics of the US Office of Education see a steady growth in bachelors degrees awarded in science and engineering up to 1985, with all the growth taken up in engineering, the life and the social sciences. But thereafter demographic trends take over. The number in the postgraduate age group (22–25) in 1995 will have dropped by 20% compared with 1985. This means not only that some inferior colleges will probably have insufficient students to justify their continuation, but also that even the big names in education will find themselves competing fiercely by 1990 to attract students to justify staffing levels.

And even before 1990 the PhD emerging from an American university is likely to find the world much changed from its present scene. Allan Cartter, in his book *PhDs and the Academic Labor Market*, projects US academic needs for junior faculty for the next twenty years. Whereas before 1970 academe could gobble up every PhD produced, by 1985 on the most optimistic projections (assuming faculty take early retirements and so on) there will only be an academic job for one PhD in every six produced in the United States. The moral is farily obvious; discourage right now those who believe three or four years research is an admission ticket to a cosy faculty post somewhere; and go on broadening the scope of the PhD so that it becomes a very widely acceptable qualification. Hence the continuing talk of interdisciplinary postgraduate education. □

Assessing the Oklo phenomenon

Professor Alvin Weinberg, Director of Oak Ridge National Laboratory, Tennessee, comments on the lessons to be learned from the discovery of a natural reactor

THE Oklo River is a small stream that runs near the town of Mounana, Gabon; here there is a large, open-pit, uraninite mine which I visited a few months ago. Oklo has become famous ever since French scientists of the Commissariat a L'Énergie Atomique discovered in 1972 that the mine was the site of ancient spontaneous chain reactors some 1.8×10^9 years ago. At that time the uranium-235 concentration was about 3%, instead of its present 0.72%. This is the same concentration now used in the fuel of modern pressurised water reactors. Indeed, the reactors at Oklo operated for about 15,000 MW years—that is, the equivalent of 5 years of a modern 1,000 MW reactor—and reached a burn-up of about 25,000 MW days per ton of uranium, again about the same as is reached in a modern PWR.

The Oklo phenomenon is surely one of the most exciting scientific discoveries of the decade. I hope that it quickly becomes part of the common understanding not only of scientists, but also of laymen—just as Pithecanthropus Erectus or King Tutankhamen have become parts of the common, as well as the scientific, understanding. I am not unbiased in this desire. Despite all the fuss about nuclear energy, I am still an unabashed nuke. For me, the long-term alternatives to nuclear energy: solar energy (and its children—wind, waves, ocean thermal gradients, biomass), geothermal energy, and fusion are either too expensive (solar) or too small (geothermal) or too uncertain (fusion).

The path of conservation is all well and good for a developed country that has energy to conserve; it has little relevance for a country such as Gabon, or for that matter, most of the world that is bent on development. Nor can I take the talk about 'small is beautiful' or a 'fission-free bridge to a solar future' fully seriously. Has anyone demonstrated on a scale large enough to make the experiment convincing, that small is really beautiful rather than simply poor? Or can anyone speak so confidently about future heavy dependence on fossil fuel with the CO$_2$ in the atmosphere increasing at a rate of about 0.15% per year, probably because we are burning so much fossil fuel? No, it seems to me that nuclear energy will be necessary *especially in the long run.* The main job of the nuclear community at present then is to make nuclear energy acceptable—to construct a nuclear future that adequately deals with the shortcomings of nuclear energy—some real, some only imagined.

We are all familiar with the issues by now: proliferation, reactor safety, terrorism and sabotage, diversion, waste disposal. Of these issues, there is little question that in the public's mind, and indeed, in the minds of most scientists outside the nuclear community, waste disposal is the most serious concern. As long ago as 1952, James Conant, the president of the American Chemical Society, asserted that the public would reject nuclear energy as not being worth the candle because the safe, permanent disposal of radioactive waste posed an insoluble problem. The recent Flowers report, on nuclear power and the environment, regards the problem of waste disposal as being in an unsatisfactory state. The new California law requires that a technology for permanent waste disposal must be demonstrated before reactors can be built there.

Until Oklo was discovered, permanent waste disposal in geologic formation seemed to be a problem that could not be solved *in principle*: since the wastes remain hazardous for thousands of years, one generation simply could not perform a definitive experiment. But Oklo has done much to change this. During the course of the chain reaction some five tons of fission products and perhaps one-half this amount of plutonium and other transuranics were formed. Of the fission products, the gases, the alkalis and alkaline earths, and ruthenium disappeared. On the other hand, the rare earths and the plutonium, uranium, and presumably the other actinides, remained in place, within the limits of measurement (at least 80 per cent of the plutonium is accounted for). The strontium-90 probably decayed in place to zirconium-90; whatever leaching of alkaline earth fission products occurred took place over a time long compared to the 30-year half-life of the strontium-90.

IAEA

The Oklo site

Thus nature has performed the experiment, and the findings, on the whole, are reassuring. To be sure cesium-137, a soluble species, migrated from the site; on the other hand strontium-90 probably did not; and most important, the actinides, which pose the really long-term hazard (thousands rather than hundreds of years) remained in place.

Sceptics will still remain unconvinced; they wil argue that Oklo represented a special geologic situation, that we cannot be absolutely sure—and that anyhow, there really is something new and spooky about this whole radiation thing. This is the essence of the matter. I shall never forget Enrico Fermi, during one of the monthly information meetings we held in 1943 at the wartime Metallurgical Laboratory, reminding us that the unique significance of the chain reaction lay in its bringing mankind in contact with unprecedented amounts of radioactivity. Never mind that radiation has become one of the best, if not the best, understood physical insults to the biosphere. It nevertheles represents something most people are unfamiliar with and are therefore afraid of.

Unless our society accepts the reality of radiation, and the proposition that radioactivity can be safely sequestered, nuclear energy will founder. And this is why it is so important that Oklo become a household word. Fermi was not the first to achieve the chain reaction, nor was he the first to put large, concentrated amounts of radioactivity on earth. Nature had achieved this 1.8×10^9 years earlier. Although the Oklo event affected life in the immediate vicinity, our planet survived and the biosphere evolved. Perhaps 100 years from now, when we have become fully accustomed to man-made chain reactors, we will look back and wonder why so much fuss was made about the problem of radioactive waste disposal which nature had already demonstrated to be tractable. ☐

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Grow your own grass

David Spurgeon examines a programme for improving agriculture in Antigua

A COMMON problem of Third World countries is an unfavourable balance of payments caused by a shortage of indigenous industry and a consequent reliance on imports from developed countries. This is particularly true of many of the small Caribbean islands, whose industries (when there are any) are often foreign-owned and more concerned with exports to an affluent overseas market than with self-sufficiency of domestic supplies.

The tiny, 108-square-mile island of Antigua is a case in point. Since a sugar cane company left the island some years ago, there has been little more than tourism and rum-making in the way of industry there—both the molasses for the rum and most of the beef for the tourists being imported. Where sugar cane once flourished, acacia bushes now grow like weeds. Yet much of this land, rocky and hilly though it often is, could be used for grazing cattle, and if only the grass and legumes were more abundant and of better nutritional quality, the island might become self-sufficient in beef and dairy production—and even export to other Leeward Islands.

This in fact is the aim of a research programme now being conducted in Antigua, Trinidad and Belize by the University of the West Indies (UWI) with the aid of a grant from Canada's International Development Research Centre. The programme began in 1972 and is now in its second phase. It has an Australian consultant, Dr Robert Burt of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), and the experiment is being watched with interest not only by the agricultural ministries of the three countries concerned but those of the neighbouring territories of Guyana, Barbados, Jamaica, Guadeloupe, Martinique and Cuba as well.

Two meetings have been held of representatives of agricultural ministries of the region—including representatives from other islands—to look at results where tests are being carried out with material from the project. The most recent was held in Antigua from 29 November–1 December. The Antigua component of the project is directed by a New Zealander, John Keoghan, who has taught at McGill's Macdonald College and done research at the University of Guelph. He is assisted by Belal Ahmed, a microbiologist from Bangladesh, employed by the department of soil science at the UWI in St Augustine, Trinidad, Clive Devers from Guyana, a graduate assistant, and Perry Phillip, a technical assistant.

Interviewed in Antigua recently, Keoghan spoke of the implications of the project and the relevance of the Australian experience. All of the grasses growing in the Caribbean and thought of as 'native' to the area, actually come from Africa, he said, while the legumes come from the area itself. The soil in both regions of the world is heavy clay, and if work done in the Caribbean were applied to Africa it could revolutionise agriculture there.

"This is not far-fetched," he said. "This is what the Australians did. They went to South and Central America and found plants for Northern Australia, which is tropical. The legumes that dominate livestock agriculture in Northern Australia were originally growing as weeds in South and Central America."

There is a difference, of course. The Australians selected plants from acid soils for acid soil areas of their own. Keoghan and his colleagues collect plants from alkaline soils to match those of the dry areas where livestock graze both in the Caribbean and in Africa. So far, they have collected a total of about 1,000 grasses and legumes for planting in experimental plots, including 70 different varieties of grasses. They are carrying out a complete classification and description of the plants, and then are making an agronomic assessment under different growing conditions, for example with different types of soils, and in mixtures of grasses and legumes.

Two plots

Two experimental plots are being used, one of them near the old sugar mill, where the small amount of laboratory equipment required is also located, along with nursery facilities.

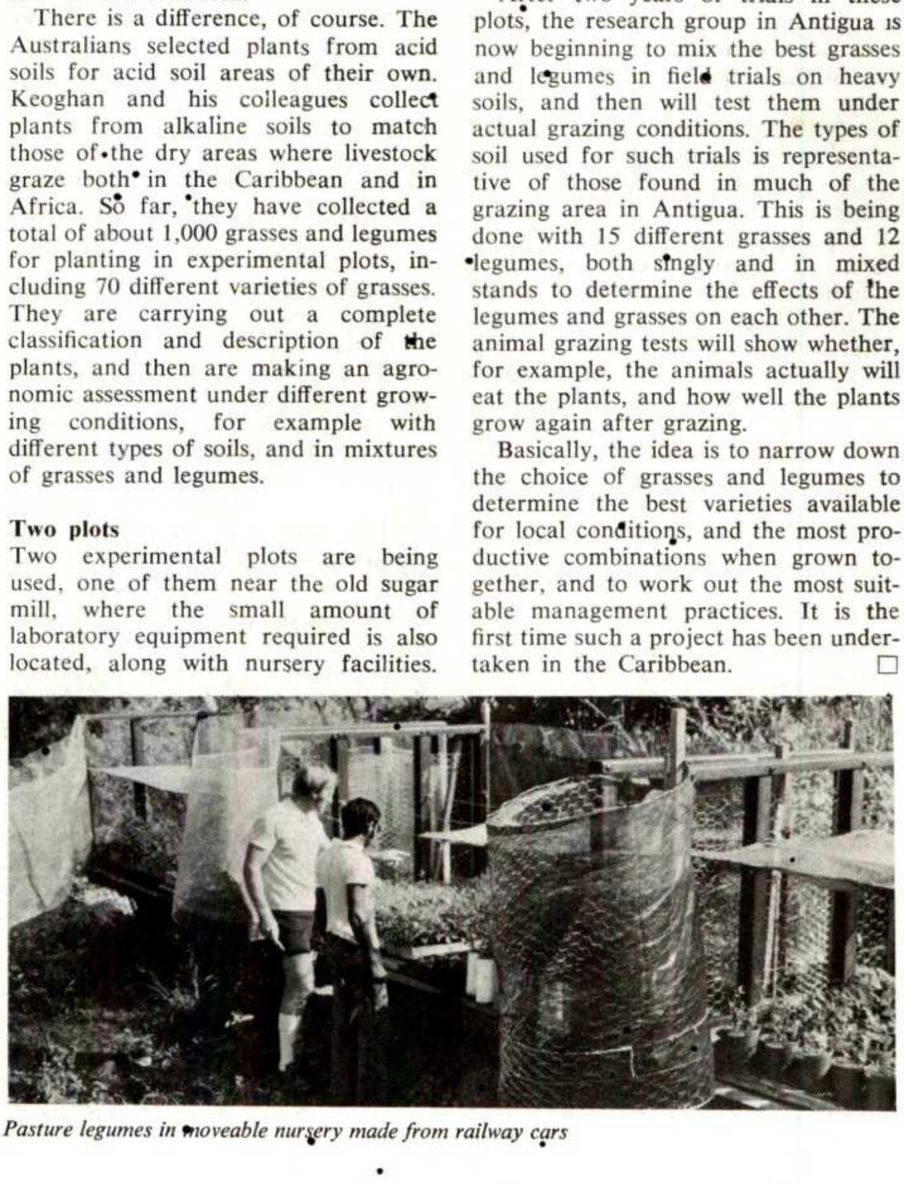
As basic as all this might seem to be, it has never been done before, according to Keoghan. Although there were earlier sporadic attempts at collection, classification and description, adequate records were not kept of them and plant collections disappeared.

Pasture legumes are important not only for their high protein content, but for their ability to fix nitrogen and thus act as a fertiliser for the grasses. This latter capability is all the more important to developing countries at a time of high fertiliser prices. Samples of legumes have been collected from all over the island as well as from other countries in the region.

Samples are selected from a six-square-metre area of each plot, the leaves are clipped and the yield is bagged, dried in ovens at the sugar factory and the weight measured. Nitrogen and protein content are determined, and records are kept of these measurements. Seeds are collected and examined, and then sent to CSIRO in Australia, which is the main centre of tropical legume research.

After two years of trials in these plots, the research group in Antigua is now beginning to mix the best grasses and legumes in field trials on heavy soils, and then will test them under actual grazing conditions. The types of soil used for such trials is representative of those found in much of the grazing area in Antigua. This is being done with 15 different grasses and 12 legumes, both singly and in mixed stands to determine the effects of the legumes and grasses on each other. The animal grazing tests will show whether, for example, the animals actually will eat the plants, and how well the plants grow again after grazing.

Basically, the idea is to narrow down the choice of grasses and legumes to determine the best varieties available for local conditions, and the most productive combinations when grown together, and to work out the most suitable management practices. It is the first time such a project has been undertaken in the Caribbean. ☐



Neill McKee

Pasture legumes in moveable nursery made from railway cars

Scottish science and devolution

Martin Goldman looks at possible effects of devolution on science in Scotland

FACED with the present media overkill of the devolution debate, more and more Scots are displaying a traditionally 'British' response to the issue—apathy. At the risk of increasing the tendency, Scottish science deserves special attention, for the problem of reconciling the international horizons of science with the nationalist viewpoint is of wider relevance, even though it is still largely theoretical. The Scottish National Party, the spearhead of the independence movement, has not yet needed to produce a concrete science policy. They have not decided whether nuclear power is an invention 'o'er the de'il', much less what role it would play in an overall energy strategy, and have not even considered whether or how to continue as members of CERN.

More positive has been the concern of academics. A strong feeling against the devolution of Scottish universities was displayed in response to a questionnaire sent out by the Scottish Association of University Teachers. About 60% of the replies disapproved of devolution; 20% approved, 20% were neutral.

A paper on the whole subject presented to the government by Professor John Gunn, a theoretical physicist, and Andrew Thomson, an economist, gives the consensus view. They feel that universities ought to be international in outlook, and therefore anything hindering communications between British universities, especially the easy exchange of staff and students, is a bad thing. The decline of the international reputation of Trinity College, Dublin, since Irish independence is an example.

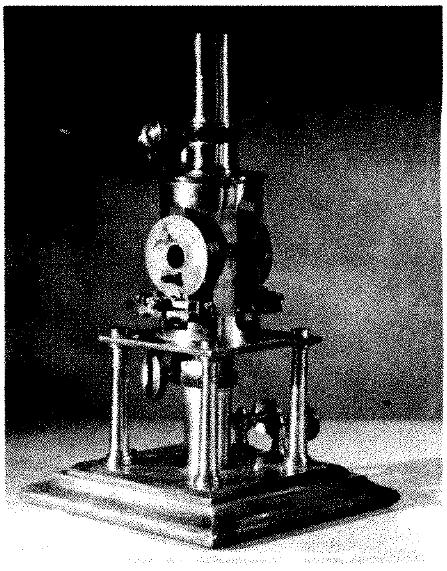
Their other major argument is that there exist economies of scale. Each university department has particular areas where it focuses its attention; Scottish universities do not cover a complete academic spectrum and are overproductive in certain areas (for example, Scotland, with 10% of the UK population, produces 150 vets a year out of a UK total of 350). Because of the expense of equipment, particularly in the sciences, and the existence of critical thresholds on the size of department, below which useful research is not done, such specialisation makes good sense.

In addition to these general arguments, they add that the present funding system, through the Universities Grants Committee (UGC) and various research councils, works well in that it allows a large degree of autonomy to the universities. If a Scottish UGC were created, it would be a cosier affair than the British one, and 'inevitable incestuous relationships' would ensue. Equally dreaded would be the alternative of integration with the Scottish Educational Department (SED) responsible for the present Scottish educational system. Universities are, of necessity, elitist organisations and are afraid they would be swamped if included with other forms of education. In addition the track record of the SED is appalling. In spite of (or because of?) a new newmaths syllabus, there has been a catastrophic disintegration of mathematical education in Scotland. All Scottish university science departments complain bitterly of the mathematical illiteracy of incoming students—the educational elite, no less—who cannot cross-multiply fractions, know no trigonometry, and require remedial courses to teach them basic skills.

Dissenting view

These views contrast strongly with those of both the SNP and the Scottish Union of Students. At the same time as Gunn and Thomson argued their case, Professor R. S. Silver, an internationally renowned expert on desalination and a life-long nationalist, presented a dissenting view. On the 'internationalist' argument, he contends that other small countries such as Holland and Israel have solved the problem. An independent Scotland would be forced to look out more to the world. He repudiates the inevitability of incestuous relationships, arguing that if this were so, then conversely Russia and China would have the fairest support system in the world. Professor Silver also points out that estrangement will inevitably arise under the present proposals between the institutes of technology (controlled from Edinburgh) and the universities (controlled from London). As for the economy of scale argument, he thinks that 'big science' has already grown too big. It leads to massive engineering projects that are socially destructive and tie together the world's economies in an unstable manner, like a house of cards.

Since then the debate has descended to the letter columns of *The Scotsman* and the *THES*. John Hulbert, prospective SNP MP and former lecturer in bacteriology at the University of Dundee, has waded in with his analysis. He finds it 'sinister' that since 1939 the proportion of professors in Scotland with a Scottish education has dropped from 73% to 48%. Remembering that Scotland has only 10% of the UK population, most would find this evidence of continued parochialism, and his views an unhappy auguration of future SNP thinking on the 'internationalism' of Scottish universities.



Produce of Scotland:
Kelvin's mirror galvanometer

To Professor Malcolm Slesser of the energy studies department of Strathclyde University, who is also a nationalist, this idea of 'technological ecology' is also central. Engineering should be on an appropriate, human scale, and vast expensive projects should be eschewed. He, for one, would not complain if an independent Scotland could not build the Concorde. Silver and Slesser's argument is weakened by the fact that whatever the final scale of a project, the cost of developing even appropriate technologies is now vast, as exemplified by the programmes afoot in the USA, France and Japan to utilise solar power. Equally, almost no science is conceivable nowadays without a big computer. How big a computer could Scotland afford?

Professor Slesser thinks the real problem of Scotland stems from a lack of financial support for the development of appropriate new technology. He would like to see a revival of the technological entrepreneurship that characterised nineteenth century Scottish science, when a scientist such as Lord Kelvin could make a fortune (and get knighted) from his inventions. An investigation of government spending supports his view. Scotland does get a fair share of the UGC's and the research councils' university spending. The research councils also support special units: the MRC supports many all over the UK, and Scotland does rather well, with 11 of these. The MRC works on the human scale, however; the SRC, which is responsible for 'big science', believes it has to have its expensive equipment housed in central facilities, shared by the country as a whole. This argument is rather damaged by the fact that its major 'centre', consisting of the Rutherford Laboratory (particle physics), the Culham Laboratory (plasma physics), the National Computer Laboratory, the High Power Laser Facility and the Appleton Laboratory (space and atmospheric research), is very conveniently situated for London, but is well south of the academic centre of the UK, which is strongly weighted by the Northern and Scottish universities.

This is a mere peccadillo compared to the policy of the biggest research spender of the country, the Ministry of Defence. Of the 45 major research establishments listed by the MOD, apart from the lonely Naval Construction Research Establishment in Dunfermline, none are farther north than Baldock, 35 miles from London. As part of the devolution package the government has promised to move the MOD to Glasgow, which has a chronic unemployment problem. Every few weeks Glaswegians read in their newspapers, with a mixture of anger and amusement, the ideas of civil servants on the putative great trek North. The most common view seems to be that civilisation stops at Chingford and the Arctic Circle begins at Watford Gap. Hopefully, if the MOD is ever moved it will learn the error of its views and devolve some of its research establishments also.

Eternal drawback

What, then, about industrial research? While the government tries to encourage industry to the North with various short term benefits, there is an eternal drawback in that Scotland is at the periphery of Europe, and transport costs must always be high. This adds about £50 to the cost of a car made in Scotland rather than Birmingham. The more valuable the basic product, however, the less significant are these transport costs, and thus factories producing high technology items are least affected. This has been recognised by the American computer giants, and Honeywell, IBM, NCR, and Hewlett Packard all have factories in Scotland. They are just factories, however, and the best Scottish managerial and research personnel are siphoned off to England or abroad. Could the process be reversed by independence? This seems doubtful, in that a small Scotland is unlikely to be more successful at dealing with a high-technology multinational than the UK as a whole.

The lack of investment in new technology is also borne out by the experiences of the National Engineering Laboratory (NEL) at East Kilbride. Seven years ago the Rothschild committee recommended that the NEL should become less academic and liaise more with industry—should become, in effect, East Kilbride's answer to MIT. To a certain extent this has worked: of an annual budget of £10,000,000, industry now contributes £1,250,000, and of NEL's marketable ideas about half are taken up by local Scottish industry—eventually. John McAllen of NEL's industrial marketing unit is, however, depressed at the lack of interest that most of industry displays in trying to identify areas where new technology could be developed. Especially depressing is the tale of NEL's hydraulically driven dumper truck. In spite of a total lack of industrial interest NEL were convinced that this idea had promise, and developed it on their own. Their perseverance paid off, and eventually Carron Hydraulics of Falkirk took out a franchise on the invention. When the potential of the idea was proved, however, Carron Hydraulics was promptly taken over by the German Rexroth group.

The story of other countries reaping the rewards from Biritish inventions is now only too familiar. The worrying aspect of this case is that the invention was developed with government money specifically to benefit British industry; safeguards against subsequent foreign takeover should have been written in. The NEL is, nevertheless, of great importance to Scotland, both as an employer of scientific personnel and as a stimulus to industry. Its status in an independent Scotland would be very doubtful. ☐

USA

Maintaining momentum

Colin Norman reports from Washington on latest developments concerning recombinant DNA research

THE debate over the risks and benefits associated with recombinant DNA research, which has been raging in college campuses and city halls around the United States, suddenly picked up momentum in Washington last week. At one end of town, a public meeting sponsored by the National Academy of Sciences provided a forum for a clash of views on whether, and under what circumstances, such research should be permitted. At the other end of town, Representative Paul Rogers, chairman of the House health subcommittee, proposed legislation to regulate all recombinant DNA research in the United States. And in suburban Bethesda, a committee of officials from several government agencies met to try to draft a bill to be sponsored by the Carter Administration.

About the only clear message to emerge from this flurry of activity is that legislation to control recombinant DNA experiments throughout the United States is now inevitable, and that it would be welcomed even by many of the scientists who have long shivered at the thought of having an agency of the federal government regulate their research. But there was the glimmering of another message, at least in the Academy's public meeting. There emerged at the meeting vocal opposition to recombinant DNA research from non-scientists concerned about its potential long-term implications for human genetic engineering. Most of the debate so far has swirled around immediate health hazards, but it may slowly be shifted toward a discussion of the potential uses to which the research may ultimately be put.

First, the legislative developments. The National Institutes of Health (NIH) guidelines published last June formally apply only to recombinant DNA research supported by the federal government, and are not backed by any federal monitoring or enforcement mechanism. Industrial research is not formally regulated at present. Because of these apparent weaknesses some state and local governments are considering adopting their own enforceable regulations. Faced with that prospect, many scientists who once resisted federal legislation would now welcome it.

Whether scientists want it or not, however, legislation is definitely coming. For the past four months a task force of some 25 officials from federal agencies has been seeking ways to extend the NIH guidelines to cover all recombinant DNA research in the United States and to monitor compliance with the guidelines. The task force has recently concluded that no existing federal agency has the authority to carry out those roles, and that new legislation is therefore needed. The task force met last week to try to draft a bill for approval by the Secretary of Health, Education and

Welfare (HEW), who would then submit it to Congress. The task force didn't complete its work, however, and it will meet again this week. Its draft legislation is expected to recommend the establishment of a licencing scheme, administered by an agency of HEW (possibly the Center for Disease Control), for research involving recombinant DNA. In any case, the controls to be enforced on the research will be the NIH guidelines.

Meanwhile, Representative Paul Rogers has already got off the mark with a bill of his own. On 8 March, he made a speech in the House outlining some of the risks and benefits potentially arising from recombinant DNA research, and announced that he would introduce legislation to control the research and that his health subcommittee would hold public hearings on the measure on 15, 16 and 17 March. The following day, he introduced a bill co-sponsored by nine other members of his subcommittee.

The timing of the move was based on political considerations. The House Committee on Science, Research and Technology announced last week that it would hold a series of public hearings on recombinant DNA research during the last week of March, and Senator Kennedy's Senate health subcommittee is planning to hold hearings early in April, after the Administration's bill is introduced into Congress. Rogers didn't want to be in the position of seeming to react to events, so he had his staff draft a bill last week in record time.

When he introduced the bill, Rogers noted that "this proposal is intended to be a vehicle for discussion . . . and does not necessarily represent the views of its co-sponsors." Its chief provisions are as follows:

- It would require the Secretary of HEW to issue regulations governing recombinant DNA research which would be "no less stringent than the physical and biological containment requirements prescribed by the (NIH guidelines)". The regulations would also specify a role for institutional review committees.
- Any researcher wishing to conduct recombinant DNA experiments would have to obtain a licence from HEW, the application for which would describe the experiment and the conditions under which it would be performed. Licences would be valid for a maximum of two years.
- The bill would limit the number of high-containment (P4) facilities in the United States to ten centres.
- It would require the Secretary of HEW to publish in the *Federal Register* a description of projects for which licences have been granted, though trade secrets would be exempted from public disclosure.
- The Secretary would be empowered to inspect facilities in which recombinant DNA research is conducted, to ensure compliance with the regulations.
- Violations of the regulations would result in civil fines of $1,000 per day.
- The bill specifies that federal regulations would pre-empt local controls on recombinant DNA research, except that the Secretary could exempt local controls which are at least as strict as the federal regulations.
- Finally, it would establish an advisory committee to assist the Secretary in the granting of licences for recombinant DNA research.

Last month a bill establishing a licencing scheme and providing harsh penalties for violations of the regulations was introduced by Senator Dale Bumpers and Representative Richard Ottinger. Senator Kennedy may also draft a bill of his own. The final version to emerge will be a compromise between many different proposals, though particular attention should be paid to the Rogers bill if only because he is in a key position to steer his proposals through the House. A recent notable addition to Rogers' subcommittee staff is Burke Zimmer-

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In an action which has so far attracted little public attention, a biologist at the University of California has been forced to destroy a large and painstakingly constructed clone bank because the clones were produced under conditions disallowed by the NIH guidelines governing recombinant DNA experiments. The incident has been described by some scientists as a good example of the way that the voluntary NIH guidelines can work to protect public health, but others have suggested that it underlines the need for more careful scrutiny of recombinant DNA research proposals before experiments are begun.

The research, carried out by John Carbon of the University of California at Santa Barbara, was performed between mid-1974 and mid-1976, a time when the NIH guidelines were under development. During most of that period, recombinant DNA research in the United States was governed by a loose set of safety guidelines drafted by a group of scientists which met early in 1975 at Asilomar, California. Those Asilomar guidelines did not rule out Carbon's experiments and even the final NIH guidelines, published on 23 June 1976, are a little ambiguous on the matter. Carbon thus did not infringe guidelines in force while he was conducting the experiments and he says he was unaware that his work lay outside the final NIH guidelines.

The work consisted essentially of constructing a large bank of clones of *E. coli* containing transplanted yeast genes and fruit fly genes. Taking the clone bank of yeast genes as an example, this, in short, is what Carbon did:—

He extracted the entire nuclear DNA from yeast cells and chopped it up into small fragments by mechanical shearing. He then spliced the yeast DNA fragments into bacterial plasmids and inserted the hybrid plasmids into *E. coli*. The next step was to culture the bacteria, growing several thousand distinct colonies, and each colony was then harvested separately. The collection of colonies constitutes a clone bank containing copies of DNA fragments, representing the entire yeast genome, spliced into plasmids inside living bacteria.

None of that would ordinarily be considered hazardous; indeed, the NIH guidelines specify that such manipulations with yeast genes require only low (P2) levels of physical containment. The problem, however, was that the *E. coli* which Carbon used to clone the hybrid plasmids also contained so-called fertility, or F, plasmids which promote the transfer of genetic information from one bacterial strain to another. This means that the hybrid plasmids contained in the *E. coli* might easily be transferred into other *E. coli* strains capable of surviving in the natural environment. In other words, use of bacteria containing F plasmids greatly lowers the barriers preventing escape of recombinant DNA molecules from the laboratory.

The scientific advantage of the system, however, is that it can be used for rapid identification of the transplanted genes, and it can also be used simply to test whether they are capable of being expressed in their new host. Essentially, Carbon transferred the plasmids from the *E. coli* bank into other strains and tested to see whether the properties of the new host were altered. In a paper published in the February issue of the *Proceedings of the National Academy of Sciences*, for example, Carbon and his co-workers report evidence that yeast genes are being expressed in their new hosts.

Last June, a couple of weeks before the NIH guidelines were published, Carbon presented the results of his work at a symposium on recombinant DNA held at Massachusetts Institutes of Technology, and his use of a system containing F plasmids was challenged on grounds of safety. Then, shortly after the NIH guidelines were issued, Carbon wrote to the NIH Recombinant DNA Advisory Committee for a ruling on whether or not his system is proscribed by the guidelines. The committee informed him last October that the guidelines should be interpreted as ruling out the use of *E. coli* containing F plasmids, and it instructed him to destroy his clone banks. Carbon was, however, given time to extract those plasmids which he wished to keep and transfer them to safer strains. He complied with the ruling last November.

Asked last week when he first had doubts about whether his experiments infringed the guidelines, Carbon said that "the realisation dawned on us slowly over the course of several weeks last spring". He said, however, that the guidelines do not specifically rule out use of his system, and he was therefore still uncertain about the propriety of his experiments even when the guidelines were issued. Other scientists queried last week said that although the guidelines are a little fuzzy on the matter, they thought that it was generally assumed that *E. coli* with F plasmids shouldn't be used. In any case, Carbon said that he is "willing to go along with the guidelines" and he destroyed more than 50,000 clones containing yeast genes and some 10,000 clones containing *Drosophila* genes.

man, a former representative of the Environmental Defense Fund who has long been active in seeking more public debate and stricter controls on recombinant DNA research.

While those legislative developments were taking place last week, the Academy's public meeting was in full, and sometimes noisy, swing at the other end of the Mall. Though the meeting essentially covered ground which has already been well trodden in college campuses and city halls around the country, it nevertheless generated considerable national attention and not a little friction.

Most of the high-temperature exchanges took place at the opening session, when a group called the Peoples' Business Commission (a reincarnation of the Peoples' Bicentennial Commission) staged a series of disruptions. Jeremy Rifkin, a spokesman for the group, was invited to address the meeting to air his grievances, and as he did so several of his supporters unfurled banners proclaiming their opposition to the research. Rifkin harangued the organisers of the meeting, claiming that the fact that it was supported financially by several drug companies rendered it suspect. In particular, Rifkin claimed that because the agenda was dominated by sessions concerned with potential health hazards from recombinant DNA research, it missed the central question of how the long-term implications of the research should be dealt with. Genetic engineering, he opined, is "the most important issue that society has to grapple with", and he told the researchers present that "you can't hide from the facts any more than the physicists who split the atom".

Although the interruptions and objections formed just a part of the meeting, Rifkin's objections did surface later in rather more subtle form, from speakers such as Jon Beckwith of Harvard, who noted that though there are still some barriers left to introducing genes into human cells, "these goals are not at all inconceivable and they may be achieved very rapidly". Beckwith announced that he has renounced use of recombinant DNA techniques, because "I do not wish to contribute to the development of a technology which I believe will have profound and harmful effects on this society".

Other speakers noted that one of the potential benefits often claimed for recombinant DNA is to help understand the causes of cancer. Such observations put the debate onto a different level from discussion of potential health hazards, and they could be much more difficult for scientists to grapple with. ☐

Sweet sorrow

AFTER more than six years of uncertainty, the Food and Drug Administration (FDA) last week decided to ban the artificial sweetener saccharin from general use in the United States. The action, which was prompted by clear evidence that the sweetener can cause bladder cancer in rats when fed to them in large quantities, immediately prompted shrill protests from the food industry, and it is sure to touch off a new round of attacks on the so-called Delaney Amendment which bans use of any food additive found to cause cancer in animals.

The evidence which finally sounded the death knell for saccharin came from a long-term feeding study sponsored by the Canadian government, preliminary results of which were made available to FDA officials on 7 March. Two days later, FDA and the Canadian Department of Health and Welfare announced that the sweetener would be banned both in Canada and the United States.

The full results of the study have not been published, but according to a summary made available by FDA, it turned up the following results. Out of 50 male and 50 female rats fed a diet containing 5% saccharin throughout their lives, three males and no females developed malignant bladder tumours. In addition, 50 male and 50 female offspring of those rats were also fed diets loaded with saccharin. The cancer incidence among them was more pronounced: 12 male and 2 female animals developed bladder tumours. Thus, 17 out of 200 test rats developed cancer compared with 2 out of 100 in a control group.

Until the full results are published, it is difficult to tell how good the study was. It has, however, long been regarded as the best test of saccharin yet undertaken, and FDA officials have been eagerly awaiting the results in the expectation that the study would finally settle the question of whether saccharin is or is not a carcinogen. They were impressed. The tests, according to Sherwin Gardner, the Acting FDA Commissioner, "show unequivocally that this substance can produce malignant bladder tumours in rats".

It has sometimes been suggested that the carcinogenic potential of saccharin may be caused not by the sweetener but by an impurity, orthotoluenesulphonamide (OTS). The Canadian tests, however, included a separate study of OTS and no carcinogenic effects were discovered. FDA was, therefore left with no choice but to ban saccharin from the market, an action which Gardner suggested was "based on science and on the requirements of Federal law". FDA hopes to publish details of the ban within a month, but it will allow those products already manufactured to be sold.

The ban could have considerable economic impact since saccharin is now the only artificial sweetener on the market in the United States. Cyclamates were banned in 1969, following evidence that they also cause bladder cancer in rats. And another sweetener, aspartame, was due to be approved by FDA a couple of years ago but approval was withdrawn following a discovery that the manufacturer of the substance submitted misleading test data. Once saccharin is removed from the market, there will be no non-nutritive sweetener for use by the burgeoning diet food industry. Some 5 million pounds of saccharin is now used in the United States, about three quarters of which goes into soda pop.

FDA's regulation of saccharin came under blistering attack last year in a study published by the General Accounting Office (GAO), an investigatory agency of the Congress. Noting that doubts about the safety of saccharin were raised in the early 1970s, particularly after a study published in 1971 indicated that the sweetener may be a potential carcinogen, GAO suggested that "extended use of a food additive, such as saccharin, whose safety has not been established and for which a carcinogenic potential has been raised, could expose the public to unnecessary risk".

FDA did, however, take some action against saccharin in 1972. It limited the amount of the sweetener which could be added to food and drink, and ordered that the substance should be used only in special dietary products. The latter requirement hasn't been rigorously enforced, however, for use of the sweetener has mushroomed, diet sodas are heavily promoted for general use, and saccharin is now found in products like toothpaste and pancake syrup.

With such a large volume of sales to protect, the food industry is expected to appeal the ban, through the courts if necessary. Some nutritionists are warning of the health consequences of an increased consumption of sugar.

Colin Norman

USSR

- The Soviet media have recently been devoting considerable attention to peaceful nuclear explosions (PNE). Although the Soviet PNE programme dates back to 1965, so far explosions have been carried out without publicity. Recently, however, a number of Soviet press and radio reports have extolled the use of PNE in digging the Pechora–Volga canal, and the construction of water and underground gas storage reservoirs. An interesting feature of this campaign is that all three major broadcasts on PNE achievements were in English (one being in the African service), and therefore aimed at the outside world; in one case, the contents of the broadcast was then issued in a *Novosti* press release.

Soviet experts do not share American doubts on the commercial viability of PNE, and are eager for the ratification of the PNE agreement, already initialled in Moscow and awaiting ratification by Washington, which would ensure mutual on-site inspection and instrumentation rights. Although the Soviet delegate to the Geneva disarmament talks, Mr V. A. Likhachev, recently called for a total cessation of all nuclear weapon tests, there are clear indications that the Soviet Union would not be willing to accept a ban or a moratorium on PNE. The recent burst of publicity may well be intended to make the Soviet attitude clear to those governments which would favour a total ban on all nuclear explosions.

- Conservation of the country's resources, including energy, is an important feature of the present Five-Year Plan. Targets include savings of 14–16% ferrous metal in machine construction, 5–7% metal, 5–6% cement and 12–14% timber in building, and 5% electrical and thermal energy and 8% petrol and diesel fuel.

This winter, inspections and surveys have been made in many parts of the Soviet Union to monitor fuel consumption. A supporting campaign in the media, commending the economical consumers and rebuking the wasteful, is aimed at all levels of the community—from the electrical generating industry, which last December cut its specific consumption by 2 g fuel per kW-hour (saving some 1.5 million tonnes of coal), to the workers of Vladivostok, who by their "thousands of valuable suggestions" have allowed some 13 million kW of electricity and 49,000 tonnes of rated fuel to be saved.

Particular criticism has been directed at the Ministry of Fuel and Power (for slowness in converting to more efficient boilers), at the metallurgical industry (for poor use of recycled resources), and the Ministry of Construction of the Oil and Fuel Industries (for delays in constructing gas refineries, so that "thousands of millions of cubic metres of gas" have had to be burned off into the atmosphere) and various local authorities (for lighting and heating empty buildings, using expensive fuel from elsewhere rather than cheap local coal, peat or wood).

- Three Moscow refusniks, the cyberneticist Aleksandr Lerner and Vladimir Slepak and Anatolii Shcharanskii, both engineers, are attempting to bring a libel suit against Dr Senya Lipavskii and the editor of the government newspaper *Izvestiya*. Dr Lipavskii, a former associate of the refusniks, is the author of an 'open letter' published in *Izvestiya* alleging that a number of the prominent Jewish activists, including the three men, are CIA agents.

Such denunciations have in the past always been followed by criminal proceedings, and it would be unprecedented if no action were to be taken in this case. Shcharanskii, who often acts as unofficial spokesman for the group, has already had all his documents except his internal passport confiscated. To try to refute the expected charges, Lerner, Slepak, and Shcharanskii have attempted to initiate proceedings for libel, although various bureaucratic barriers are being placed in their way.

Together with the economist Ida Nudel and Dina Beilina, an engineer, they have also written a letter to President Carter, asking him to do something "constructive" to ease the situation. Although the text of the letter has not been released by the signatories, it seems that they would urge a firm but 'soft' response. The letter of Academician Sakharov to President Carter last January, which called on the President to "raise your voice" against human rights violations in the Soviet Union, was interpreted by the Soviet authorities as a provocation to cold war, as was the recent meeting of the President with Vladimir Bukovskii. Lerner and his friends, it would seem, are unwilling that their case should be used to undermine the very real benefits of détente.

- The new Soviet 200-mile fishing zone, which came into force on 1 March, has inaugurated a new era in Japanese–Soviet fishing relations. So said the Japanese Minister of Agriculture and Fisheries, Zenko Suzuki, during four days of stiff negotiation in Moscow at the turn of the month. By the end of the talks it was clear that the new era was not to be one of immediate agreement; indeed, Mr Suzuki and the Soviet Minister of Fisheries, Aleksandr Ishkov, could not even agree on a joint communiqué, and the talks ended in a formal exchange of notes.

During the talks Mr Suzuki stated that for constitutional reasons Japan could not accept certain provisions of the decree establishing the Soviet zone. His concluding note to Ishkov stated that Japan would also be establishing a 200-mile zone in the near future. This will encroach upon the new Soviet zone on the west coast of Japan and in the region of the La Pérouse and Kunasharskii straits. In spite of these diplomatic difficulties, however, certain practical conclusions were reached, pending the interim pact (expected by 31 March). Japanese fishing vessels will be permitted to operate within the new Soviet zone, but may not engage in herring or salmon fishing. Negotiations are to be continued towards a long term agreement.

A similar position, but with the Soviet Union in the role of the applicant seeking to enter an exclusive zone, has arisen in the fisheries talks between the Soviet Union and the EEC. The first round of talks, in February, were hailed as a diplomatic breakthrough; the second round failed this month to produce the "mutually acceptable results" which Mr Ishkov had hoped for. Since both sides realise that they are at present too far apart to reach an early agreement, the talks have been postponed until 19 April.

Vera Rich

BRITAIN

• Figures on graduate employment from three of Britain's top universities don't conform with everyone's predilections when it comes to science graduates.

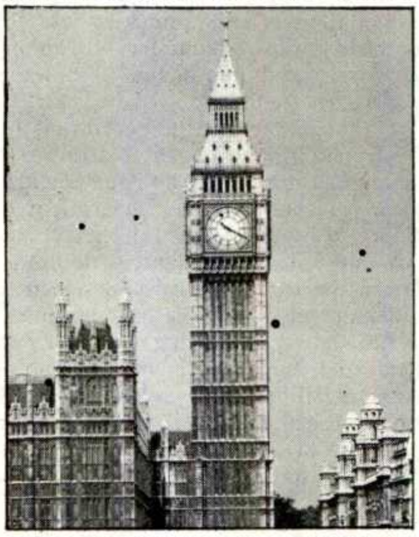
The view heard these days is that graduates, especially from Oxford, Cambridge and London, are likely to go into the types of employment which served Empire well but which are of less obvious use in the modern industrial era. The suggestion is that they are most likely to go into central and local government or teaching when they aren't busy becoming perpetual students by doing post-graduate study. Otherwise they are likely to be joining the professions—becoming accountants or solicitors, moving into banking or insurance—or joining the media by going into journalism, broadcasting or publishing. What they aren't doing, the argument goes, is helping to boost Britain's long term economic potential by going into directly productive employment in industry.

Figures giving details of employment of 1976 science graduates from Oxford, Cambridge and London do not appear to bear these assertions out. Even after making allowances for difficulties in interpreting the statistics in precisely comparable terms, the indications are that Britain's graduates in the pure sciences, if they are not going into research, are most likely to go into industry.

That they are likely to go on to further study is strongly indicated by the figures for both Oxford and Cambridge, where a high proportion, around one-third, of the men science graduates from both places opted for this course. But of the 390 Oxford men science graduates who worked in other fields in Britain after graduating, 155 went into industry; and of the 193 Cambridge science graduates, 88 did so. For London, the figure was 209 out of 497.

For the public service and education, moreover, the figures are not high. Just 20 out of 193 from Cambridge went into the public service, and 23 directly or indirectly into teaching. The comparable figures from Oxford are 65 and 44 (out of 390) and from London 75 and 68 (out of 497). Similar tendencies are apparent in figures for accountancy (32, 58, 47) and for banking and insurance (7, not available, and 34). And from all three universities together a total of only 21 men went into the media or elected to become solicitors.

What makes the figures even more interesting is that similar trends, though less marked, can be identified for arts and social studies graduates. At the other end of the university machine, meanwhile, the number of entrants into tertiary education who go into science and engineering remains a prominent topic, not least, it seems, because the facts are disputed. At the beginning of the month the Education and Science Secretary, Mrs Shirley Williams, described the need to attract students into science and engineering courses as "urgent". Her department is at the moment investigating whether to introduce a form of scholarship to encourage sixth-formers to specialise in science and engineering. But the Vice-Chancellor of Durham has warned of a glut of engineers, and the London University Institute of Education is saying the shortage of engineers and scientists is a myth.



• The state of Britain's manufacturing industry is an issue unlikely to be superseded in importance by questions about her agriculture, itself an industry. At the end of last month, though, it was announced that the country's standing Royal Commission on Environmental Pollution, now under the chairmanship of Professor Hans Kornberg, was to examine how the trend towards large livestock units and the greater use of fertilisers and pesticides posed an increased risk of pollution. The Commission also wants to examine the vulnerability of agriculture to industrial pollution.

Among the bodies which might show an interest is the Nature Conservancy Council (NCC). A report it published last week suggests that financial assistance and advice on conservation should be given to farmers if the problems their farming practices create for wildlife are to be alleviated. The report estimates that if all farms were to be totally modernised, about 80% of the bird and 95% of the butterfly species would be lost from the farmed landscape. The NCC wants a policy of wise land use which provides for the conservation of wildlife as well as increased food production.

Increased food production was just one of the points broached last week by Professor John Bowman, the Director of the Centre for Agricultural Strategy at the University of Reading, when he spoke about an agriculture strategy for Britain. He talked of food consumption ("comfortably in excess of or equivalent to recommended minimum levels for normal life"), of changing eating habits to help feed others less fortunate (that had "little to commend it"), curtailing animal production, because of worries about its cruelty or its energy inefficiency ("it is more justifiable to argue for the modification of production systems so they are based more on feed inputs of no direct use to man"), and justifying protectionist policies in terms of security (there are sound arguments for achieving security "through an increase in the world trade of all commodities, not least of which are food commodities").

He also presented the preliminary findings from the Centre's own model of the UK agricultural economy. Its projections for a future with a higher degree of self-sufficiency and lower import costs indicated broadly that, even allowing for modifications in diet, meat production was expected to contract, the dairy sector, sugar beet and oilseed rape were expected to expand, and the cereal acreage would remain largely unchanged. But technical and economic efficiency were unlikely to be the only objectives of public policy; justice, equity and security could take precedence.

• The University of Bradford's Disaster Research Unit, which was established in December 1973 and has just three members, is now fighting for its survival. When the initial period of three years for which the unit was financed came to an end late last year, the university (which provided the funds along with the Leverhulme Trust and the Ministry of Overseas Development) agreed to finance a further year pending the outcome of a review. This showed that with the cuts it faced the university could not support the unit. Now the unit has stopped work while its members ask the government, international agencies and trusts for more funds.

Chris Sherwell

Potential energy

Developments on Britain's energy front are continuing apace. Chris Sherwell reports

GAS, coal, oil and nuclear power have all featured in the past week's energy developments. At the risk of involving the Cabinet and angering the unions, the Energy Secretary, Mr Anthony Wedgwood Benn, is proposing to overrule the Price Commission's rejection of the British Gas Corporation's (BGC) application for a price rise. The rise, effective next month, will boost BGC revenues by 10% and is part of the attempt to rationalise Britain's energy industries.

The development came after news that additional coal resources amounting to more than 200 million tonnes have been discovered in Warwickshire. The seams compare with the Selby and Vale of Belvoir fields, and are the result of the National Coal Board's exploration programme. A new £50 million three-year programme is due to start soon, and more discoveries are expected.

But not everything is rosy. Coal prices are also going up next month; NCB projections of output until the 1980s under its Plan for Coal show that exploitation of new mines is beset by problems in obtaining planning approval; and productivity shows a downward trend.

On the oil front the important matter of depletion policy in the North Sea is attracting attention The issue emerged clearly recently in the Department of Energy's broad review for the NEDC. An annex stated that large-scale licencing in the early 1970s was designed to build up an exportable surplus in the 1980s; delays in starting work on new fields and "slippage" in the build-up of commercial fields, however, had "somewhat eroded the level of production expected in the early 1980s and therefore the export surplus".

Last week a slightly more optimistic prognosis for the export surplus was produced by a policy adviser to the British Petroleum Board, who counselled against a strict depletion policy designed to prolong the period of self-sufficiency. The issue looks like becoming increasingly important as the time for critical nuclear power decisions draws closer.

Arguably the most important of those decisions concerns the fast breeder reactor. One report this week says there are suggestions for a supervisory board, representing all concerned interests, to manage the project; an executive team of experts would report to it. The Prototype Fast Reactor at Dounreay, due to be shut down recently for a ten-week overhaul and for refuelling, will meanwhile take over the role of the small experimental reactor, which will be closed in a ceremony next week after some 17 years of service In the reprocessing field Mr Benn's intervention last weekend has seen the end of the six-week industrial dispute at the British Nuclear Fuels plant at Windscale ☐

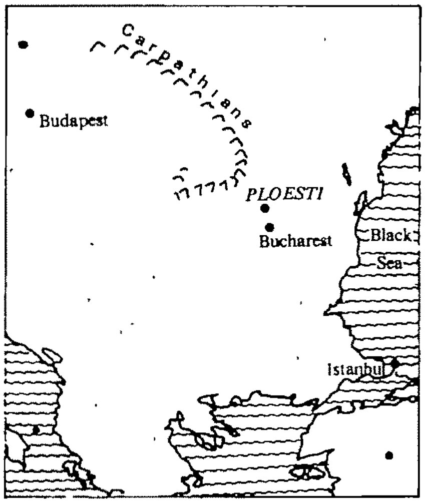
Quake patterns

BACKGROUNDER

EARTHQUAKES in Romania have many unusual features to them, not least a somewhat erratic occurrence rate. The recent quake of magnitude greater than 7 near Ploesti, which has caused such widespread damage and the loss of over a thousand lives, seems to conform to the pattern.

There is regular seismic activity in a band running down the east coast of the Adriatic, through the Aegean Sea and off into Northern Turkey, but further north, activity is rare, suggesting that the major active plate boundaries run well south of Romania. There is, however, a little activity along the north coast of the Black Sea, and a small isolated pocket of Romanian quakes lies along the continuation of this line at the southern most limit of the Carpathian mountains. These quakes are at a depth of 50 to 150 km and lie in a vertical slab (*Nature* **228**, 1176–8 (1970)); the slab is probably the relic of a small segment of ocean that was overridden millions of years ago but is still out of equilibrium with its surroundings. The earthquakes would then result from the release of stress as the slab settled.

Since the recent quake there have been several attempts to discern patterns in the times of occurrence of Romanian earthquakes Rarely can credence be attached to such statements which have been made on the basis of a rapidly compiled historic compilation. Although the catalogues back to about 1900 are quite good for large earthquakes, the estimate of magnitude for many of these events leaves a lot to be desired; any analysis which then looks at the statistics of events above a certain magnitude is bound to be using shaky premises.



Even so, the suggestion emerging from the US Geological Survey (and regrettably inflated into an ambassadorial message) is of interest. It has been noticed that six of the eight quakes since 1900 which the Survey regard as large have occurred in pairs, separated by a month or so. This is quite an unusual observation for earthquakes, and the prospect of a second large quake cannot be discounted. Prediction techniques based on rock dilatation that are beginning to emerge as important for shallow quakes seem difficult if not impossible to apply at present to events as deep as the Romanian quakes.

The earthquake was felt over a very wide area—Moscow, a thousand miles away, was shaken slightly. This had been noted for previous shocks and probably arises from a combination of two factors. First, an earthquake at depth, away from the near surface inhomogeneities, irradiates a larger area than does a shallow shock. And second, there is probably very little absorption of elastic waves crossing an old, stable region such as Western Russia.

Legal proceedings, it is reported, are to be brought against architects whose modern buildings collapsed in the quake. There is a certain amount of sense in this. An earthquake of comparable dimensions occurred in 1940 and was destructive; it seems that in some cases appropriate lessons have not been learnt. Earthquake-proofing of buildings is by no means an impossibly expensive task, and is done routinely in California and Japan, with great success Maybe one of the good things to emerge from the disaster will be a greater insistence on building to safer standards.

IN BRIEF

Science budget breakdown

The Department of Education and Science (DES) was unable to comment this week on a report in *THES* detailing the breakdown of the Science Budget for 1977–78. The figures have yet to be agreed formally with the Treasury and passed by the House of Commons.

The Science Research Council's budget is believed to break down as follows: nuclear physics, including the CERN subscription, £43.3 million; astronomy, space and radio, £27.6 million; science, £23.3 million; engineering, £14.5 million. Figures for the other research councils are reported to be as follows: MRC, £42.3 million; NERC, £29.5 million; ARC, £20.4 million; SSRC, £14.5 million. The size of the overall budget was foreshadowed in the recent expenditure White Paper.

NASA change

James C. Fletcher, who has been head of the National Aeronautics and Space Administration (NASA) for the past six years, is being replaced, another casualty of the Carter Administration. He announced last week that he will leave on 1 May. The post is a Presidential appointment and Fletcher consequently submitted a pro-forma resignation to President Carter along with other holdovers from the Ford Administration, but it was no secret that he wanted to be kept on. No reason has been given for accepting his resignation and no replacement has been announced.

UN conference opens

Experts from some 150 countries were Argentina, this week for the opening of the two-week UN Water Conference. The major item for discussion highlighted last year's Habitat conference in Vancouver: providing clean and safe water supplies to residents in urban and rural areas as soon as possible. Also on the agenda: pollution control, conservation of water resources, management of shared lake and river resources, water as a disease carrier.

Psychiatrist's death

The death has been recorded of Professor Daniil Lunts, head of the fourth section (political) of the Serbskii Institute of Forensic Psychiatry.

Last weekend a young paramedic, Aleksandr Podrabeinnik, who has collected details of over 200 cases of sane mental hospital internees in the Soviet Union, had his flat searched and papers confiscated.

Monster debate

THOMAS H. JUKES

THE debate on recombinant DNA is so far a tribute to the power of the human imagination rather than an acknowledgment of actual results obtained in laboratories. Most of the stronger statements seem to be made by people who are not trained in biology. However, some good ammunition has been provided by prominent scientists. For example, Robert Sinsheimer recently advanced the possibility that DNA from *Clostridium botulinum* might be used by a 'terrorist group' to transform *E. coli* into producing botulinus toxin. Obviously, such a group will not apply for a permit from the National Institutes of Health.

The environmentalist societies have reacted promptly and swiftly to the issue. The decision was easy, because in matters of science and technology, especially new ones, they are guided by the principle stated by Groucho Marx in the 1930s:

> I don't know what you're going to say
> It makes no difference anyway
> Whatever it is, I'm against it!

Some of the busiest recombinant DNA laboratories are in our own digestive tracts, where the transducing phages, episomes and Hfr strains have been switching DNA around ever since the days of early evolutionary history. Knowledge of this should (but won't) have a calming effect on those who believe that recombination is a recent invention of maniacal molecular biologists.

I am impressed above all by the fact that nature buries her countless numbers of mistakes. The two-headed calf does not survive. Nor does nature help us in our genetic manipulations. The most productive strains of our major crop plants would rapidly vanish without human protection and nurture. Maize would be the first to disappear. It is true that there are deadly pathogenic micro-organisms in our midst but the number of species of these is comparatively small. They are the product of millions of years of commensal evolution with their hosts. The thought that we could slap new ones together in the lab and that they would be viable enough to run riot seems faintly ridiculous.

Even such an apparently obvious advantage as antibiotic resistance does not easily persist. Some years ago I participated in launching an experiment that spread widely; the addition of antibiotics to the feed of domestic livestock. Although this has recently been criticised and scrutinised, the strange fact remains that it 'still works', when by all predictions the spread of resistant strains should long ago have made the procedure useless. This is especially so because resistance in enteric bacteria can spread by DNA recombination. I presume the wild, susceptible strains must tend to overgrow the resistant ones.

Public understanding of recombinant DNA is greatly aided by previous conditioning to science fiction. A long-time staple subject for cartoonists in popular magazines is typified by a picture showing 'mad scientists' in a laboratory where enormous rats are emerging from a flask, captioned 'Something Went Wrong'. This theme, almost to the letter, was used in a recent cartoon, and, to make the point absolutely clear, the scientists wore lab-coats labelled 'MIT'.

So far, the one predictable result of 'recombinant DNA research,' even the research that hasn't been done, is a bureaucratic explosion of paperwork. Any mention of DNA in a research grant proposal presses the alarm button, and a large package of questionnaires arrives in the mail. We can probably look forward to a new National Institute for Regulating Recombination, and even to its counterparts in state and local governments. Also, the term 'recombinant DNA' rolls trippingly from the tongue, and politicians know a vote-getting issue when they see one.

correspondence

The toxicity of plutonium

SIR,—We wish to comment on three recent publications, all originating in the United Kingdom, which have discussed the radiotoxicity of inhaled, insoluble plutonium compounds. In a recent article in *Nature*, Thorne and Vennart[1] concluded that the previously accepted value for the maximum permissible annual intake (MPAI) of such compounds may be too high by a factor of about five. A similar conclusion had previously been reached in a Medical Research Council report[2], and in a report[3] by the Royal Commission on Environmental Pollution. Our main purpose here is to point out:

- that the proposed changes in the MPAI and in the closely-related maximum permissible air concentration (mpc) do not necessarily imply any change in the present procedures for assessing doses and risks in situations where persons have known amounts of insoluble plutonium in their lungs;
- that although the article in *Nature* and the MRC report come to broadly the same final conclusions, the reasons for the conclusions are significantly different; and that the uncertainties in the calculations, to which attention is drawn in both papers, are as large as the proposed reduction factor;
- that the observed behaviour of the technologically-important high fired plutonium oxide in people who have accidentally inhaled such material is significantly different from the behaviour predicted from the lung model assumed in recent MPAI calculations.

Maximum permissible annual intake. Thorne and Vennart stress that the MPAI which they derive is intended for planning purposes[1]. We believe that most facilities in which plutonium oxide is handled are planned on the basis that there will be virtually no chronic exposure, and that this is also true in operational practice; hence the precise value of the MPAI (or the mpc) is somewhat irrelevant. However, exposures do occur from time to time, due to unplanned but identified incidents which usually cause the exposure of one or two individuals to quite high concentrations for a short time. In such situations, we believe that it is good practice to make the best estimates of intake, dose and risk to those specific individuals on the basis of measurements taken on them, with no reference to the concepts of MPAI (or mpc). In fact, such a procedure is explicitly recommended[4] in *ICRP Publication 19* on the page following the one on which they describe the model used in *Nature* and the MRC report.

Uncertainties in the MPAI. Although the *Nature* article and the MRC report both conclude that a reduction in the MPAI of the order of a factor of five is to be recommended, the two sets of calculations show significant differences. For example, the respective estimates of risk of bone cancer differ by more than a factor of five and the risks of lung cancer by more than a factor of three. On reading the respective texts it is obvious where the differences lie (in the relative importance of the respiratory lymph nodes, in the quality factor for alpha particles and in the calculations of dose to the bone). Both publications acknowledge that the estimates of 'nominal risk' are open to criticism and that the data on which they are based can be interpreted in several ways. It is an unfortunate fact that such qualifications are often disregarded by those who draw conclusions from the final tabulated presentation.

The maximum permissible lung burden (MPLB). In the summary of conclusions (paragraph 526.2) of its sixth report, the Royal Commission on Environmental Pollution[3] claimed that a reduction by a factor of five in the MPLB is already practised. This statement appears to have resulted from a misunderstanding; we believe that their statement was intended to apply to the MPAI. The conclusion stated in paragraph 526.2 does not follow from the section of the report in which the matter is discussed (i.e. paragraph 77). Neither the MRC report nor the *Nature* article explicitly suggested a reduction in the MPLB, and in our view, no such reduction is required in the case of high fired plutonium oxide.

ICRP lung model. As explained above, all of the recent calculations have been based on the use of the lung model of *ICRP Publication 19*. The parameters given in the model were derived mainly from animal data, and presumably represent at best the mean or median values of a number of widely differing experimental values. Consequently, as explained in the associated text of the ICRP publication, the model can only be used for deriving general exposure limits; to evaluate a specific exposure situation one should choose the data most relevant to that particular situation. Measurements made on a substantial number of individuals[5,6], who had inhaled high fired plutonium oxide, show that the material initially deposited in the lung is removed much more rapidly than the ICRP model would suggest for what it calls a Class Y material. Moreover, experience here at Winfrith (to be published) has shown that the amount of material transferred from the lung to other parts of the body is also significantly less than the model would suggest; perhaps this is a consequence of the more rapid removal to which we have referred. As a consequence of these two factors, if risk estimates are made for those people who have inhaled high fired plutonium oxide, they are found to be a factor of about 5 lower than the risk estimates based on the use of the lung model, i.e. they are no higher than was previously supposed.

Discussion. We feel that the methods which have been used to derive new values for the MPAI are unduly pessimistic for high-fired plutonium oxide. That fact in itself does not worry us unduly. What does worry us is that people may be tempted to conclude, by the use of the same lung model, that in every case in which a person inhales insoluble plutonium, the risks to bone and liver are comparable to those of lung; and hence to conclude that the MPLB should be reduced. We believe that in the case of high fired plutonium oxide, the previously established idea that the lung is the critical organ remains substantially correct.

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[1] Thorne, M. C., and Vennart, J. *Nature* **263,** 555–558, 1976.
[2] *The Toxicity of Plutonium* (HMSO, London, 1975).
[3] *Sixth Report of the Royal Commission on Environmental Pollution* (Chairman Sir Brian Flowers, Cmnd. 6618, HMSO, London, 1976).
[4] *ICRP Publication 19: The Metabolism of Compounds of Plutonium and the Other Actinides* (Pergamon, Oxford, 1972).
[5] Ramsden, D. *Diagnosis and Treatment of Incorporated Radionuclides,* 139–161 (International Atomic Energy Agency, Vienna, 1976).
[6] Watts, L. *Health Physics* **29**, 53–59, 1975.

Registering Russian mail

SIR—Apropos of your article "Keeping in touch with Soviet colleagues" (10 February, page 484), it is my experience that correspondence with them is expedited by using registered mail.

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Culham Laboratory, UK

news and views

Marburg and Ebola: viruses in search of a relation

from David I. H. Simpson and Arie J. Zuckerman

IN the second half of 1976 two severe epidemics of haemorrhagic fever broke out in Sudan and Zaire. The virus responsible, provisionally named Ebola virus, proved to be structurally similar but antigenically different from that of Marburg disease, and three recent reports in *The Lancet* give details of its isolation and preliminary characterisation.

Marburg virus

Marburg virus disease, popularly but incorrectly named 'green monkey' disease, is a severe distinctive haemorrhagic febrile illness of man, first described in 1967 when thirty-one cases of illness with seven deaths in Germany and Yugoslavia were traced to direct contact with blood, organs or tissue cell cultures from a batch of African green monkeys (*Cercopithicus aethiops*), which had been trapped in Uganda. Several secondary cases occurred in hospital personnel by contact with the blood of patients, and one further case was apparently transmitted by sexual intercourse 83 days after the initial illness. The case fatality rate was 29% for the primary cases, but there were no deaths in the six secondary cases. It soon became apparent that this was a previously unrecognised disease caused by an infectious agent probably new to medical science.

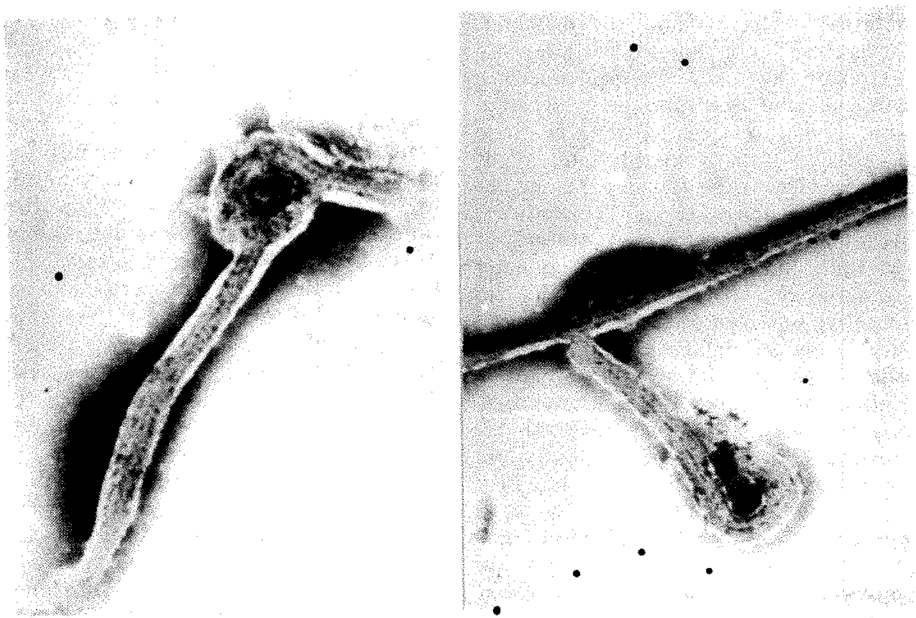
The possible origin of the infection has been investigated since the 1967 outbreak. Complement fixing antibodies to the Marburg virus were reported from a laboratory in the USA in many African monkeys, but conflicting results were obtained in other surveys and it appeared that the antigen used in the USA was not entirely specific. Furthermore, before 1967 many thousands of monkeys had been handled in biomedical laboratories without such outbreaks, and in addition experimental infection of monkeys proved uniformly fatal. The natural cycle of transmission of the virus in various species of animals and the origin of the infection are still under intensive investigation.

The first recognised outbreak of the disease in Africa and the first since the original outbreak, occurred in South Africa in February, 1975. The primary case was a young Australian man who had hitch-hiked through Rhodesia. He died in a Johannesburg hospital and shortly afterwards his girl travelling companion and one of the nurses who had cared for him fell ill with the same disease. The girls recovered. Virological studies showed that this outbreak was caused by the Marburg virus.

As in the original Marburg outbreak, when virus was isolated from seminal fluid 83 days after the onset of illness, evidence was obtained that the virus can persist in the body for at least 2–9 months after the initial infection. In the Johannesburg episode, virus was cultured after infection of the eye from fluid aspirated from the anterior chamber of the eye 80 days after the onset of the illness (Gear *et al. Brit. med. J.* **4**, 489; 1975).

Ebola virus

Three recently published papers by Pattyn, Bowen and Johnson and their colleagues (*Lancet* **i**, 569–574; 1977) now give details of the isolation and preliminary characterisation of the virus responsible for two severe and almost simultaneous epidemic outbreaks of haemorrhagic fever in the equatorial provinces of Sudan and Zaire in the second half of 1976. The isolates were found to be structurally indistinguishable from strains of Marburg virus


Electron micrograph of the Zaire prototype strain of Ebola virus (courtesy of E. T. W. Bowen and W. J. Harris).

isolated in Germany in 1967 (Siegert *et al. Dtsch. med. Wschr.* **92,** 2341; 1967) and South Africa in 1975, but antigenically the Sudanese and Zaire isolates although identical to each other were quite distinct from Marburg. The prototype of these new strains has been named Ebola, after a small river in Zaire which flows north of Yambuku, the village of origin of the patient from whom the first isolate of the virus was obtained. Experimental studies in laboratory animals (E. T. W. Bowen, unpublished results) confirm the antigenic differences since previous immunity to Marburg virus does not protect against subsequent infection with Ebola virus and *vice versa.*

Morphology

Marburg and Ebola viruses show no antigenic relationship to any other animal virus and morphologically they belong to no known virus group. Marburg virus contains RNA, has an outer envelope containing lipid, and is ether sensitive. Both viruses are formed by budding through cell membranes and it has been suggested (Siegert *et al. op. cit.*) that Marburg resembles members of the rhabdovirus group. However, the size of the central core and the cross-sectional structure differs from that of rhabdoviruses.

Two principal structural forms of Marburg virus have been described (Almeida *et al. Marburg Virus Disease* (eds Martini & Siegert) 85, Springer-Verlag, Berlin, 1971): an elongated filamentous or sinuous form, which could be described as tubular; and a circular form in the shape of a 'doughnut' or torus.

Both forms have an overall diameter of 70–100 nm excluding projections on the outer surface and contain an internal helix 40 nm in diameter. Several of the sinuous particles are extremely long, often several microns. Almeida and her colleagues suggested that as mature virus particles give rise to circular torus forms Marburg might be regarded as the founder member of a group known as the Toroviruses. However the predominant forms of Marburg and Ebola most frequently seen in the electron microscope in negatively stained preparations (Figure) are long tubular structures and since they contain RNA perhaps a better name might be Tuburnaviruses, a name suggested at an informal discussion between K. M. Johnson and ourselves. Geographical names for diseases or their causes are also to be discouraged since these may be misleading, implying as they do an unusual association with a particular country or town, and may then lend a sinister conotation to the area, as in the case of Lassa, a town in North-Eastern Nigeria and Marburg, a University town in Germany.

Epidemiology

It has now been generally accepted that monkeys are not the natural reservoirs of Marburg infection. Intensive epidemiological investigations in Southern Africa following the three cases in Johannesburg in 1975 failed to provide many clues to the natural maintenance host of the virus. Similar investigations in Sudan and Zaire have not yet uncovered the reservoir of Ebola virus. The Zaire and Sudan outbreaks involved well over 500 cases and at least 350 deaths. The illness was marked by a sudden onset of severe headache, fever, muscle pains and prostration, quickly followed by profuse diarrhoea and vomiting and later in many severe cases by dramatic haemorrhages, and closely resembled the illness described in Germany in 1967 and in South Africa in 1975. The typical skin rash so common in white-skinned patients was not so distinct in black skins. In each outbreak, be it Marburg or Ebola fever, attendant medical and/or nursing staff became infected often with disastrous results. Two hospitals in Zaire and Sudan were brought to a standstill; in one hospital in Sudan 76 staff became ill and 41 of them died. Direct human-to-human transmission was responsible for all the secondary infections usually brought about through close and prolonged contact with sick patients and particularly through contact with patients' blood.

The fact that Marburg and Ebola viruses once they have been transmitted to man through this contact with an unknown reservoir host can adapt themselves to direct man-to-man transmission means that these severe infections could easily be introduced from their country of origin to countries where the natural host does not exist. Hence the international concern over these infections and others such as Lassa fever. It is therefore all the more important that detailed epidemiological investigations are carried out without delay. □

Trypanosome antigens

from F. E. G. Cox

INFECTIONS with African trypanosomes are long-lived and are characterised by waves of parasitaemia in which the parasites seen in the blood at any one time differ antigenically from those of preceding waves. This antigenic variation is thought to be responsible for the prolonged infections in what ought to be immune hosts because, although the hosts make antibody to each antigenic variant, they are foiled when the antigens of the trypanosomes change and each becomes effectively another parasite. The mechanisms of antigenic variations are interesting from both theoretical and practical viewpoints and over the past few years three important questions have been asked. First, are variant antigens situated in the surface coat, second, if so, are they produced by the trypanosomes or are they accretions of host serum proteins and, third, do all trypanosomes carry a range of variant antigens or do certain individuals possess one kind of antigen and others a different kind? In the case of *Trypanosoma brucei* these questions have now been satisfactorily answered.

It is now known that the variant antigen actually constitutes the majority of the surface coat of the trypanosome (Cross *Parasitology* **71,** 393; 1975; Cross & Johnson *Biochemistry of Parasites and Host Parasite Relationships* (ed. van den Bossche, H.) 413; 1976) and that it is a glycoprotein consisting of a single polypeptide chain of molecular weight 65,000. The different variants are not simply minor modifications of a basic type because when the amino acid sequences of related variants are analysed they are found to be completely different from one another (Bridgen, Cross & Bridgen *Nature* **263,** 613; 1976). So the answer to the first question is that not only are the variant antigens associated with the surface coat, they actually form much of it and furthermore the successive variants represent structurally dissimilar polypeptide chains. Could these have come from the host serum? A recent paper by Taylor and Cross (*Parasitology* **74,** 47; 1977) proves that they do not. When trypanosomes are maintained *in vitro* in the absence of host material variant antigens are still synthesised and incorporated into the surface coat. This confirms the original hypothesis of Vickerman (*J. Cell Sci.* **5,** 163; 1969) and refutes the suggestion by Desewitz (*Immunity to Parasitic Animals* **2,** (eds Jackson, G. J. *et al.*) 551; 1970) that the variant antigen is assimilated from the host.

Trypanosomes revert to a basic antigenic type after they pass through the tsetse-fly vector and the subsequent variants appear in a recognisable sequence. This occurs even in infections initiated with single parasites (clones) and this has been interpreted as meaning that trypanosomes do not exist as mixed populations with different individuals expressing different variants but actually continually change their antigens. However, experiments by van Miervenne *et al.* (*Annl Soc. belge. Med. trop* **55**, 1, 55, 25; 1976), using a fluorescent antibody technique to identify particular variants, have shown that clones rapidly become antigenically heterogeneous and that in every population there are a few individuals that do not bear the antigen characteristic of the population. These individuals are at an advantage when the immune response to the predominant antigen begins to become effective but are in their turn destroyed as they become dominant and have to give way to yet other variants.

Cross and his coworkers suggest that trypanosomes express a gene for and synthesise a single variant antigen at a time. Over evolutionary time a number of genes each coding for a particular variant have emerged by mutation, been selected for and have been accumulated giving the trypanosomes of the present time a vast repertoire of variants which they need to survive in their mammalian hosts. None of the available evidence contradicts this hypothesis and the next questions must be how the antigen switch is brought about and, when this is known, how it can be stopped. To those living and trying to raise cattle in the tsetse belt of Africa this is not just an academic question. ☐

Prenatal detection of thalassaemia

from Sue Malcolm

THE various inherited haemoglobin disorders which collectively go under the name of thalassaemia are the most serious genetic diseases in many parts of the world. So the successful prenatal diagnosis of both the α and β thalassaemias marks a significant advance in their prevention.

α thalassaemia (in which no α globin chains are produced) is now known to be caused by the deletion of the DNA coding for the α chain (Ottolenghi *et al. Nature* **251**, 389; 1974; Taylor *et al. Nature* **251**, 392; 1974), and Y. W. Kan and colleagues in San Francisco have now devised a method of determining whether the α globin genes are present in foetal DNA (*New Engl. J. Med.* **295**, 1165; 1976).

The foetal DNA was extracted from cultured amniotic fluid cells obtained by amniocentesis during the fifteenth week of pregnancy and cultured for a further five weeks to provide enough DNA for analysis. Radioactively-labelled α globin complementary DNA synthesised on α globin mRNA was then hybridised to the foetal DNA. The results from this technique are quite clear cut but require the greatest precision in mixing the two DNAs and care in making the measurements.

At the ratios of cDNA to DNA used there should be three α globin minus strands (two from the cold foetal DNA and one from the cDNA) to every two plus strands in normal DNA, and therefore the radioactive probe should be 66% hybridised. If two α globin genes are deleted (α thal I) the ratios would be two minus strands to one plus strand, and only 50% hybridisation found, and if three genes are deleted (Hb H disease) the minus : plus ratio would be 3 : 1 and 33% hybridisation found. If in fact all four α genes are deleted in the foetus (hydrops fetalis) no hybridisation would be expected. Unfortunately the α globin cDNA was only 80% pure, the remaining sequences being β cDNA so even in a case of hydrops fetalis around 20% hybridisation would be found. A successful diagnosis therefore rests on the ability to distinguish between 20% and around 45% hybridisation ($\sim$125 c.p.m.) since only the difference between hydrops fetalis and Hb H is clinically important. In the case reported Kan successfully predicted that the foetus examined had α thalassaemia I. Later, twins were diagnosed and the parents had no hesitation in continuing the pregnancy since at least one foetus was known to be unaffected. In the event healthy twins, one male and one female, were born. At least some of the uncertainty in this type of diagnosis will be removed when cloned, and therefore pure, human globin sequences become available.

An even more serious health problem is posed by the diseases associated with faulty β globin chain production, β thalassaemia and sickle-cell anaemia. Reports from a combined Boston and London study (Alter *et al. New Engl. J. Med.* **295**, 1437; 1976) and from Kan's San Francisco group (*Lancet* **i**, 269; 1977) announce encouraging progress in the prenatal diagnosis of both these diseases. In both cases there is an error of β globin expression rather than a gene deletion; in β thalassaemia no β globin chain is produced and in sickle-cell disease a mutant globin chain(β_S). Therefore analysis can only be carried out on cells which actually produce globin—the foetal blood cells.

The biochemical problem has been elegantly solved. The blood cells are incubated with ^{3}H-leucine and the resultant globin chains analysed on carboxymethylcellulose chromatography columns. In the case of β thalassaemia the β/γ globin ratio is measured and in the case of sickle-cell disease the presence of β_A or β_S is determined. Prenatal diagnosis is possible because even in the first trimester of pregnancy 10% of the β-like chains produced are β rather than the foetal γ globin.

Serious problems in interpretation could arise if the blood sample were contaminated with maternal cells. The Boston–London group overcame this problem by transfusing the mother to reduce her reticulocyte (globin-producing cell) count before foetal blood sampling and the San Francisco group used anti-i serum, an antibody that agglutinates foetal cells. Both groups detected homozygous β thalassaemia and homozygous sickle-cell disease *in utero* and their results were confirmed after abortion.

However, both groups also found foetal blood sampling a hazardous procedure and altogether 6 out of 55 foetuses were lost as a result of attempting to sample foetal blood (three from each centre). The Boston–London group used placental aspiration when there was an anterior placenta and foetoscopy for a posterior placenta. The San Francisco workers used placental aspiration in both cases. The Boston-London workers found foetoscopy less hazardous and since their very first attempts they have had far greater success. The one case in which they wrongly predicted a healthy baby was the result of an insufficient foetal blood sample.

Although the results of the two groups combined are very encouraging it is clear that attempts to take foetal blood during early stages of pregnancy can be very dangerous and the continuing use of these methods for prenatal diagnosis depends on this procedure's becoming much safer. As pointed out in an editorial in the *British Medical Journal* (**531**, 532; 1977) it will also be necessary to simplify the biochemical procedures and the apparatus involved before these techniques can be applied in the parts of the world where these diseases are common. ☐

Polarity time scale extended by 2 Myr

from Peter J. Smith

WITH the possible exception of any very short events remaining undetected, the details of the geomagnetic polarity time scale based on radiometrically-dated subaerial igneous rocks of the past 4.5 Myr have been known for some time. It would now clearly be desirable to extend this well-established scale backwards, partly to increase its usefulness as an aid to stratigraphic correlation and partly to reduce the errors involved in matching it to much longer polarity reversal patterns (for example, from oceanic magnetic anomalies and deep-sea sediment cores) based largely on relative dating. Unfortunately, however, this is far from easy. For one thing, with increasing age it becomes more and more difficult to find rocks which remain unaltered and thus not subject to geological 'errors' such as loss of radiogenic argon. But even where suitable material is available, beyond 4–5 Myr, potassium–argon ages have precision errors comparable to many of the time intervals to be resolved.

One way out of the latter dilemma, as McDougall *et al.* (*Geol. Soc. Amer. Bull.* **88**, 1; 1977) have found, is to concentrate on a long stratigraphically controlled sequence of rocks and rely on mathematical techniques to smooth out data points which individually would not be sufficiently precise to define polarity boundaries. The sequence that McDougall and his colleagues chose comprised more than 400 successive lavas in Borarfjördur, western Iceland, with a total thickness (including interbedded sediments) of more than 3,500 m. The palaeomagnetism was straightforward; most of the lavas gave reliable directions and thus polarities and a reversal pattern. The sucess rate for potassium–argon analysis, on the other hand, was much lower. In fact, only 24 samples were considered suitable for dating on the basis of minimal alteration of primary mineralogical phases and minimal development of secondary minerals.

The selection of rocks for radiometric dating is admittedly still largely subjective. Although fresh well-crystallised lavas are generally suitable and highly altered lavas are obviously useless, there is particular difficulty in deciding whether or not only slightly altered samples should be rejected, especially where (as in Iceland) completely unaltered rocks are so rare that little progress could be made with them alone. In this case, however, there were ways of assessing the overall reliability of the results obtained. For example, the 24 potassium–argon ages determined by McDougall *et al.* were generally consistent with the stratigraphy, suggesting them to be fairly good indicators of the ages of crystallisation of the lavas rather than of the age of any subsequent burial metamorphism. Indeed, they showed no correlation at all with the positions of the rocks with respect to Iceland's well-mapped zeolite zones.

So accepting that their measured ages were "valid estimates, to a first approximation, of the time since the lavas were erupted", McDougall and his coworkers then proceeded to carry out a simple linear regression analysis of determined age against the aggregate thickness of the lava–sediment sequence measured from its base. Notwithstanding the implicit assumption here that the sequence accumulated at a uniform rate throughout, the best-fitting age-thickness curve gave an age of 3.27 Myr for the clearly-recognisable Gauss–Gilbert boundary. This is in good agreement with the well-established age of 3.32 Myr for this boundary, suggesting that both the basic data and the assumptions made in analysing them were valid. Even so, to normalise the data a new regression analysis was carried out using only rocks older than the Gauss–Gilbert transition fixed at 3.32 Myr.

The revised regression line together with the polarity data give an age of 5.34 Myr for the Gilbert–Epoch 5 boundary, 5.83 Myr for the Epoch 5–Epoch 6 boundary and 6.54 Myr for the Epoch 6–Epoch 7 boundary. The Cochiti and Nunivak normal events within the Gilbert reversed epoch appear as a single event lasting from 4.09 to 3.86 Myr ago—a failure of resolution possibly arising from a hiatus in the eruption of lavas at the critical time. However, the two normal events in the lower Gilbert, termed C_1 and C_2 by Opdyke (*Rev. Geophys. Space Phys.* **10**, 213; 1972), appear separately, covering the periods 4.45–4.33 Myr and 4.81–4.60 Myr, respectively. The names 'Sidufjall event' for C_1 and 'Thvera event' for C_2 are now proposed.

As for older epochs, the unnamed reversed event previously recognised within normal Epoch 5 by several groups of workers is now seen to cover the period 5.50–5.37 Myr whereas the unnamed normal event previously seen within reversed Epoch 6 covers the period 6.39–6.27 Myr. But McDougall *et al.* also find two further normal events within Epoch 6—very short ones at 6.43 Myr and 6.12 Myr. Apparently these have not been detected previously either in marine magnetic anomalies or in deep-sea cores.

No doubt some of these ages (which have uncertainties of 0.1–0.2 Myr) will be subject to amendment by subsequent workers; it would be surprising if that were not to be so. In the meantime, however, McDougall and his colleagues have made a major contribution in extending the polarity time scale based on subaerial rocks back about 2 Myr further than previous attempts. Incidentally, the extended scale now covers the Miocene–Pliocene boundary for which McDougall *et al.* propose a revised age estimate of 5.2±0.1 Myr although admitting that there may be data (for example, from New Zealand) inconsistent with this figure. ☐

Earthquake prediction

from E. R. Lapwood

The Fifth US–Japan Seminar on Earthquake Precursors, sponsored by the Japan Society for Promotion of Science and the National Science Foundation, met in Tokyo on 17–19 January 1977. It was organised by Professors Z. Suzuki and C. Kisslinger and the proceedings will be published as a special number of the *Journal of The Physics of the Earth.*

Hopes that monitoring of the ratio of speeds of sound and shear waves in stressed rock would be the key to earthquake prediction have been disappointed, but Japanese and American seismologists believe that successful prediction will be achieved. When, however, will depend on the intensity of effort, on the level of funding and on effective international cooperation.

In both Japan and the US the budget directly provided for earthquake prediction research runs into millions of US dollars. (The 1976 US programme was for $5 million, and the Japanese—much greater—for $8 million not including salaries, Seismologists in both countries are hoping for increased support in 1977.) Especially in Japan this earmarked budget is to be seen against a background of highly efficient routine observations and widespread research in universities. Papers presented to the seminar showed that understanding of earthquake origins and mechanisms is advancing on a wide front; and that that is essential in view of the complexity of the problems and the variety of possible premonitors.

Experimental work on the behaviour of rocks (and other materials) was described by W. Brace (Massachusetts Institute of Technology) and K. Mogi (Tokyo University). Brace emphasised the important differences in behaviour of intact rock and 'gouge' (the crushed material created at the rough interface by sliding on a fault), in particular as regards dilatancy (expansion just before breakdown) and its accompanying effects. At a huge fault with a history of repeated movements in recent time the layer of gouge may be very thick, whereas at a fault with no such character it may be thin; thus the response of the material just before slip on the fault, and premonitory signals, may be very different. Mogi described his experiments on dilatancy of rocks under triaxial stress. Previous experimental work had been mainly with axial symmetry. When the three principal stresses are unequal the response is more complicated and more realistic, so that Mogi was able to describe effects very relevant to earthquake mechanics.

The importance of Japanese records often emerged. For instance W. Thatcher (US Geological Survey) reported that analysis of peat layers in a trench cut across the San Andreas fault enabled movements in the past 1,500 years to be listed, giving an average interval of 170 years between large local movements. But T. Matsuda (Tokyo University), using Japanese historical records, was able to classify all active (that is, Quaternary) faults in Japan according to estimated slip rate and recurrence time, and to set upper and lower bounds on the greatest earthquake to be expected on a given fault at a given time. Again, the recent discovery of anomalous uplift between 1960 and 1974 in Southern California has led US agencies to allocate $2.1 million for its observation and study. In Japan, on the other hand, 20,000 km of levelling has been covered five times since 1884 (except for Hokkaido) and geodesists are already in a position to detect anomalous uplifts in any part of the country. At present the Tokai region (southwest of Tokyo) has been designated as an "area of intensified observation", partly because of increased crustal movement since 1974.

E. R. Engdahl (NOAA) described the results obtained by himself and C. Kisslinger (University of Colorado) from two high-gain high-frequency seismograph networks in the central Aleutians. The high level of seismic activity and the precision with which epicentres within the net, and depths of foci, could be located produced splendid maps and cross sections, by means of which the migration of activity could be followed, and the pattern of foreshocks and aftershocks of a moderate (m_b5) earthquake examined in remarkable detail. Equally impressive were the results achieved at the Observation Centre for Earthquake Prediction at Tohoku University. A. Takagi (Tohoku) showed graphs of the hypocentre (focus) distribution of intermediate-depth micro-earthquakes below the North-East Japan arc. He demonstrated a double-plane structure, sources lying on parallel dipping planes separated by 30–40 km.

S. Suyehiro (Japan Meteorological Agency) described the close coverage of the Japanese islands with seismograph stations, and plans for telemetering and for emplacing ocean-bottom seismographs which in a few years should enable the hypocentres of earthquakes of magnitude $M>3$ to be computed in real time (as the earthquakes happen). Fault–plane solutions, describing the source mechanism, are routinely computed for events of $M>5$. Local seismograph networks, operated by various universities, are able to see the fine structure of hypocentres among foreshocks, aftershocks and seismic swarms.

But neither these agencies nor any other in Japan or the US has been able to identify regularly occurring seismic precursors in the form of anomalous velocity ratios or P delays. Only for compressive stress in hard rock—and not always then—do such precursors appear. Moreover, Japanese seismologists have a good deal of evidence that dilatancy is confined to crustal rocks. T. McEvilly (University of California, Berkeley), in a review of the present state of knowledge of precursors, gave the following rough estimates of thresholds above which a long-term coherent anomaly could be distinguished from noise (Table 1).

He considered that it was not yet possible to give a similar table for expected values of precursors; deformation precursors are well documented, but there is serious difficulty in predicting the time of the main event; other physical and chemical parameters have not yet been shown to give consistent indications; animal behaviour should, if it is significant, lead to measurable physical parameters.

Much interest was aroused by the paper by S. Suyehiro and H. Sekiya (Japan Meteorological Agency) on anomalous seismic activity (not classed as foreshocks) before seismic events, and by the paper by C. Scholz (Columbia University), on a possible deformation front moving across North China at about 110 km yr^{-1} at the time of the earthquake at Hai-cheng (Liao-ning province).

Having given a pessimistic estimate of the possibility of compiling during the next 10 years enough case studies of large earthquakes of different types to make prediction of destructive earthquakes feasible, F. Press (Massachusetts Institute of Technology) appealed for the pooling of resources in instruments and men for a cooperative effort from China, Japan, USA and USSR—the countries which now exert 90% of relevant worldwide research effort.

Z. Suzuki and C. Kisslinger (University of Colorado), joint organisers of the symposium, pointed to the rising public demand for earthquake prediction, and emphasised the need for seismologists to learn to speak intelligibly to ordinary people. H. Ohta (Tokyo University) and E. Haas (University of Colorado) reported surveys of social attitudes and responses; from these it was very clear that the education of the public to understand statements of probability and to respond rationally must precede announcements predicting earthquakes. In fact, it must begin at once in countries at seismic risk. □

Table 1

Differential magnetic field	1γ in 10 km
Electrical resistivity	1% change in 10 km
Electric field	1+% change in 10 km
Atmospheric electric field	? 200%
Radon content in wells	20% in a few months
Water level in wells	Depends on local conditions
Strain	1 in 10^6
Tilt	1 microradian
Uplift	
Levelling	1 cm in 10 km
Gravity	3×10^{-8}g
Differential sea-level	2–5 cm
Travel times of seismic waves	
Relative P delays	
(beyond 40° and between stations within 50 km)	0.1 s
V_p/V_s ratio	
(over periods of months, with good processing)	0.1
Velocity changes from local earthquakes	1%
Controlled sources	
(explosion, air-gun, vibrator)	10^{-4}–10^{-3}

Optical fibres at Williamsburg

from J. Baker

The Second Conference on Optical Fibre Transmission was held at Williamsburg on 22–24 February, 1977.

THE effort in fibre optics communications technology (the transmission of information by light pulses travelling down hair-like fibres of glass) is concentrating on realising on a practical level the inherent advantages of the system—high bandwidth, immunity from electrical interference, small size, and low cost.

Many organisations reported the results of field tests on complete information-carrying networks suited to a variety of applications. N. Shimodaira (Nippon Electric Co.) reported the results of field tests on a high bit rate (400 Mb s^{-1}) optical link, the transmission of such high bit rates being made possible by two factors. First, the gradient of the refractive index in the fibre is carefully built up during fabrication, decreasing smoothly from the centre to a constant value in the "cladding". This results in nearly equal group velocities for the various propagating modes which each pulse of light excites. Second, the modes excited by the pulses of light are restricted to those of low order, thus further reducing the total spread. Ga–Al–As semiconductor laser diodes were used to launch pulses into the fibres, and avalanche photodiodes were used to detect them at the receiving end. At the other end of the spectrum W. H. Hackett (Bell Laboratories, Murray Hill) reported the results of tests on short-haul low bit-rate (16 and 32 Mb s^{-1}) data links incorporating light-emitting diodes to launch the pulses and PIN photodiodes to detect them.

Information transmission by way of optical links has inherent advantages in environments where high electric fields are present. T. Sekizawa (Fujitsu Laboratories Ltd) reported the results of a cooperative venture which has demonstrated this convincingly in field conditions. They found that the performance of their optical fibre links, which were routed through ducts containing power cables, were not measurably degraded by proximity to high voltages.

Several papers were devoted to splicing techniques. The contribution by P. K. Runge (Bell Telephone Laboratories, Holmdel) and W. C. Young (Western Electric) was notable, the connector described being moulded directly to the protective sheath of the optical fibre. The authors quoted losses per connector in the region of 0.4 dB. However it was clear that more work is necessary before a viable splicing system becomes available for general field use.

In the field of fibre fabrication a great deal of effort has been devoted to producing consistently very strong fibres. Both C. Kao (ITT Electro-Optical Products Division) and C. R. Kurkjian (Bell Laboratories, Murray Hill) quoted breaking strengths in excess of 500 k.p.s.i. for 125 μm diameter fibre by ensuring that surface defects and contamination of the fibres during manufacture were kept to a minimum. Fatigue strengths of fibres under applied stress were also reported which indicated that potential service lives greater than 20 years can be expected for fibre now being produced.

The overall impression gained was that optical communications systems are now a viable proposition for capacity data links. Concerning the future for long haul telecommunications, the question is now how soon?

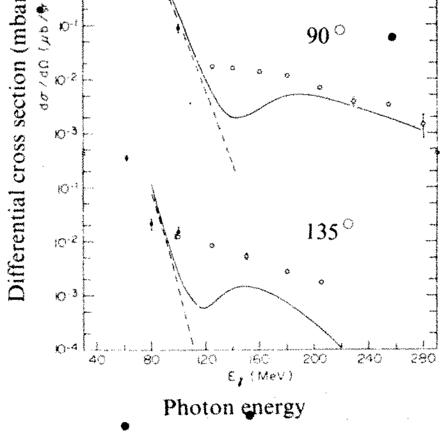
Short range nuclear forces

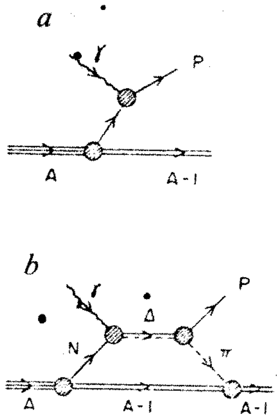
from P. E. Hodgson

EXPERIMENTS on the photo–proton reaction on oxygen from 100 to 300 MeV by a joint MIT–Glasgow group have recently provided evidence that in this energy range the reaction proceeds through the Δ (1232) nucleon isobar as an intermediate state, and it is likely that this process is increasingly important at high energies (*Phys. Rev. Lett.* **38**, 8; 1977).

The experiment was carried out by bombarding a beryllium target with a bremmstrahlung beam and measuring the spectrum of the emitted protons. The differential cross sections for the photo–proton reactions on oxygen that leave the residual nucleus in its ground state are shown for several angles as a function of photon energy in Fig. 1.

It is simplest to suppose that the reaction proceeds by a single-step process in which the incident photon interacts with a single proton in the target nucleus and knocks it out. This is indicated diagrammatically in Fig. 2*a*, and gives the dashed curves in Fig. 1. It is clear that this theory, allowing for uncertainties in the calculations, is in qualitative accord with the data at low energies, but quite fails at higher energies. The calculations are rather rough, since harmonic oscillator wave functions were used, and the distortion of the wave function of the outgoing proton was neglected, but this does not affect the general conclusion.





The experimental cross sections at higher energies are much higher than those given by the one-step process. Such cross sections can be attributed phenomenologically to short-range correlations due to the repulsive core of the nucleon–nucleon interaction or a microscopic model can be made in terms of a two-step process. One such process, involving the Δ (1232) nucleon isobar excited in an intermediate state, is indicated in Fig. 2*b*. Calculation with this process gives the full curves in the figure, when added to the results of the calculations of the one-step process already considered. Although there are differences in detail due to the approximations made in the calculation, it is clear that the overall trends are qualitatively given. This provides evidence that the Δ-process can make a major contribution to the photo–proton cross-section in the 100–300 MeV region, and thus gives further insight into the structure of the short-range nuclear forces. ☐

A hundred years ago

THE Balloon Commission of the French Government, styling itself "Commission pour les Communications par Voie Aérienne," has become a standing institution, and includes within its province carrier pigeons as well as aeronauts and balloons.

From *Nature* **15**, March 15, 439; 1877.

review article

Some recent developments in remote sensing

P. Schagen*

In recent years electronic instruments have been developed which increase very considerably our ability to view the nocturnl scene at otherwise critically low incident light levels, by using the available reflected radiation more efficiently with the aid of image intensifiers. Still other instruments employ sensors which are sensitive in the infrared and convert the natural thermal radiation of any object in the scene into visible pictures. In this article recent technological developments in image intensification and thermal image conversion are briefly discussed.

REMOTE sensing depends on a large variety of techniques and finds application in a wide range of human interests. The techniques used may be 'passive' methods of obtaining information about a particular parameter of the environment, for example its local variations in emission or reflection of natural electromagnetic radiation. They may be 'active', using probing beams of light (as in laser radar systems and range finders) or irradiating the scene with X rays or ultrasound to obtain pictures for medical diagnosis or non-destructive testing.

It is impossible in a brief article to present a comprehensive survey of the field as a whole. Instead, the discussion has been restricted to some recent developments in two methods of passive sensing of the environment, using image intensification and thermal imaging. Both are of particular importance at very low levels of incident illumination.

Perception at very low light levels

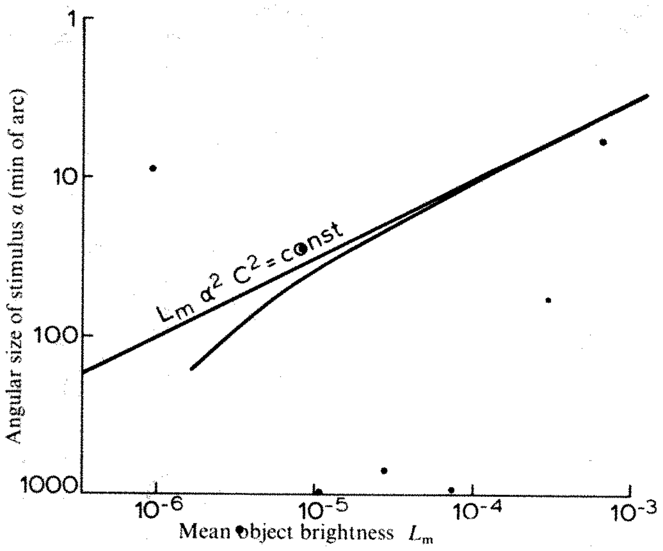
In daytime the visual acuity of the eye is limited only by the retinal cone density and the optical aberrations of the eye lens[1,2], because the level of photon flux is far greater than the level of its statistical fluctuation. On the other hand, at night when incident light is reduced to starlight, airglow and occasionally moonlight, the numbers of photons captured by the eye are so small that their statistical fluctuations become the limiting factor. The eye will only perceive an object detail in the scene if it can detect the difference in illumination of two corresponding adjacent elementary areas on the retina. Each of these areas registers a number of photons, on average, during the integration time of the retina. The difference between these average numbers constitutes the photon 'signal', with an associated photon 'noise', caused by the statistical fluctuations in the numbers during successive integration periods. If the actual numbers of detected photons become so small that the photon-signal is indistinguishable from the photon-noise, then the eye is not able to sense the object detail.

As light level decreases and photon-noise becomes an important factor, the eye tries to compensate for the reduction in the density of photons received in a number of well-known ways. First, the pupil dilates to let the eye capture more photons from the scene—the pupil diameter is 0.2 cm at a brightness of 10^4 cd m^{-2}, but rises to, and levels off at a diameter near 0.8 cm when the brightness is down to 10^{-4} cd m^{-2}. (10^4 cd m^{-2} is roughly the brightness of white paper on a bright summer's day; 10^{-4} cd m^{-2} is roughly the brightness of a cloudy moonless sky at night.)

Second, dark-adaptation takes place: the cones, the sensor elements of the retina which are active in daylight and are responsible for colour vision, cease functioning at a scene brightness below about $10^{-\text{[illegible]}}$ cd m^{-2}, and the rods, which are overloaded in daylight and which are not colour-sensitive, slowly recover and take over. An important aspect of this process seems to be the ability of the neurones associated with the rods to integrate the light signals produced by the rods over larger areas of the retina as the illumination level falls. The minimum signal-to-noise ratio required for the detection of a picture detail can thus be maintained during a reduction in scene brightness by several orders of magnitude for correspondingly larger areas on the retina, leading to a gradual decrease in resolving power.

The third effect on the eye of a reduction in scene brightness is that the retina integrates the signal over longer time intervals,

Fig. 1 Typical acuity curve of the eye for a bright stimulus on a black background, together with a straight line expressing the relationship of equation (1).



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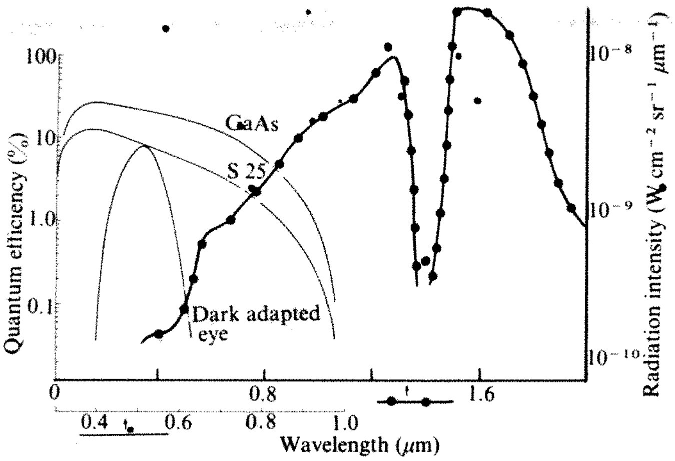


Fig. 2 Typical spectral response characteristic of a dark adapted eye in comparison with a multialkali photocathode of the S25 type, and with a GaAs photocathode[10,11]. The quantum efficiency curve for the eye is based on the relative energy response curve of ref. 3, p. 73, while assuming a maximum quantum efficiency of 8% at 0.507 µm, as calculated from equation (1) and Blackwell's measurements. Added (●–●–●) is a typical spectral distribution of night sky radiation.

up to about 0.2 s, thus again achieving the minimum signal-to-noise ratio required for perception from correspondingly dimmer details, but now at the expense of perceiving movement. A critical flicker frequency can be defined which is linearly related to the logarithm of the stimulus brightness over a wide range of brightness values. This effect is still noticeable at much higher illumination levels, such as that of the fluorescent screen of a television receiver, where an irritating 50-cycle flicker can become apparent if the screen brightness is set too high.

The performance of the eye, like that of any image-forming instrument, can be described with the aid of acuity curves, relating the resolving power (and thus the detection range) to brightness and contrast on a logarithmic scale[3,4]. If photon noise is the fundamental limitation at the lowest light levels, then these acuity curves can be represented there by relations of the type[5]

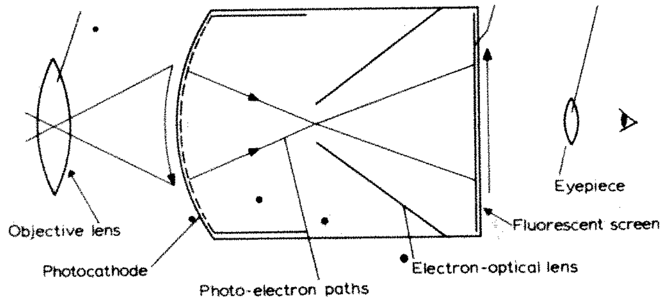
$$L_m\alpha^2C^2\tau = \text{constant} \qquad (1)$$

where L_m is the mean brightness ($L_m = \frac{1}{2}(L_{high} + L_{low})$ in cd m^{-2}) of two adjacent object elements forming the detail with an optical contrast C, defined as $C = (L_{high} - L_{low})/(L_{high} + L_{low})$, α, the acuity, is the minimum angle (minutes of arc) subtended by each of the two object elements at which their difference in brightness can still be detected, and τ is the integration time.

The constant in the right-hand side of equation (1) can be written as

$$\frac{7.5 \times 10^{10}(S/N)^2_{min}}{D_{eff}{}^2\,\theta P}$$

Fig. 3 Schematic diagram of an image intensifier tube.



where D_{eff} is the effective diameter (cm) of the objective lens of the instrument, θ the quantum efficiency of the sensor and P the number of photons per second equivalent to 1 lm of the radiation employed; both θ and P depend on the spectral distribution of the light (1 cd m^{-2} = π lm m^{-2} on a white surface). $(S/N)_{min}$ is the minimum signal-to-noise ratio required for detection of the object detail. Its value depends on the kind of object detail which the detector must resolve. For some test objects the value of $(S/N)_{min}$ can sometimes be determined mathematically with the aid of probability theory[6], by considering the number of alternative choices available and the reliability required in the decision. Blackwell[7], for instance, has measured acuity curves of the eye by asking observers to look at a circular disk, flashed on to the screen for a short period in any one of eight positions, and requiring a reliability of 50% in the answer. For this situation it can be shown that $(S/N)_{min} \sim 1.5$.

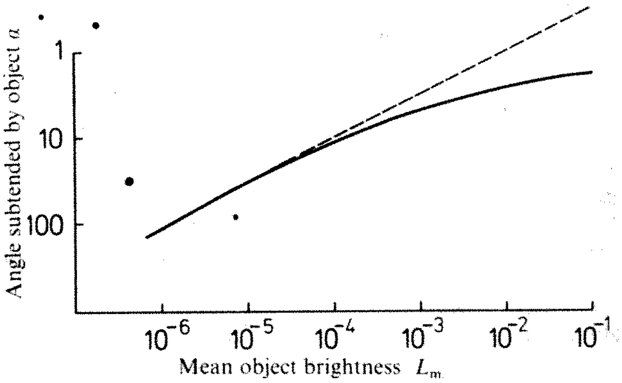


Fig. 4 Schematic acuity curve (arbitrary units) of an image detector. The dashed line is the equation $L_m\alpha^2C^2\tau$ = const. At low brightnesses the detector is photon-noise limited; at high brightness the optics limits resolution.

In Fig. 1, a typical acuity curve of the eye is plotted from Blackwell's data[7] for a high-contrast (C = 1) bright stimulus on a dark background. The curve closely approximates the straight line of the photon-noise limited condition, indicated by equation (1), over several orders of magnitude of the light level L_m, with a value of $L_m\alpha^2$ equal to approximately 10^{-2}. If the diameter of the dilated pupil is taken to be 0.76 cm, and a binocular summation factor of 1.26 is assumed[8] to take into account the use of both eyes in Blackwell's experiments, then D_{eff} can be taken to be 0.85 cm. So for τ = 0.2 s and $(S/N)_{min}$ = 1.5, Blackwell's data indicate a quantum efficiency of the dark-adapted eye of just below 1% for 'white' light with 1 lm equivalent to about 1.3 × 10^{16} photons s^{-1}.

At 0.507 µm, where the dark-adapted eyes of young people have their maximum, 1 watt is equivalent to 1766 lm (ref. 9). This leads to P = 1.45 × 10^{15} photons s^{-1}, indicating a maximum quantum efficiency of the dark adapted eye at 0.507 µm of about 8%, according to equation (1). To improve further on the capabilities at low-light levels we may turn to image intensifiers.

Image intensifiers

Instruments of this kind collect more photons from the scene with the aid of a larger lens diameter (up to tens of centimetres) than that of the dark-adapted eye. They use these photons more efficiently, by means of sensors with a larger quantum efficiency than that of the retinal receptors and a spectral sensitivity extending further into the infrared region, as shown in Fig. 2. By so doing they take advantage of the rapid rise in intensity of night-sky radiation (also shown in Fig. 2).

The sensors are image intensifier tubes, first suggested in a patent in 1928 (ref. 12) and illustrated in Fig. 3. The output image can be several thousand times as bright as the image projected on the cathode. An important condition is that the brightness gain is sufficiently high to ensure that an electron created by a photon at the photocathode gives rise to so many photons on the fluorescent output screen of the tube that it will be detected by an observer even if not fully dark adapted.

The performance of the instrument can again be described with the aid of acuity curves, similar to that of Fig. 4, approaching the photon-noise limitation at the lowest light levels and limited by its optical imperfections, as measured by its optical modulation transfer function[13, 14], for the smaller picture details. The main improvements over the unaided eye at low light levels are due to the much larger product $D_{\rm eff}^2\theta P$ in equation (1).

Two types of image intensifier tube can be considered for modern night viewing equipments, both providing sufficiently high brightness gain to satisfy the condition formulated above. The first, now well established in production instruments, uses so-called cascade tubes[15], illustrated in Fig. 5. Single-stage image intensifier tube modules are stacked in cascade, using fibre-optic coupling between the output screen of one module and the photocathode of the next. Each fibre-optic window contains millions of glass fibres with diameters of less than 10 μm. Up to three modules are encapsulated together, complete with a miniaturised wrap-around high-voltage supply unit.

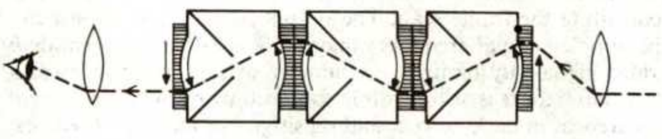


Fig. 5 Principle of operation of a three-stage cascade tube.

High brightness gains can also be achieved by channel electron multiplication[16–19], illustrated in Fig. 6. A hollow tube or channel is shown, usually consisting of a glass with suitable electrical conductivity. When a potential is applied between two end electrodes an accelerating electric field is established down the length of the channel. An electron entering the cathode end of the channel strikes the wall and releases a few secondary electrons. These will be drawn further into the channel under the influence of the accelerating field, and strike the opposite wall at a higher potential, thus releasing further secondaries. The process is repeated several times, giving rise to a large bunch of electrons like an avalanche emerging at the exit for one initial electron.

Since the electron gain depends critically on the length-to-diameter ratio of such a channel and the voltage applied, it is possible to make the individual channels very small and stack them together in a mosaic or channel-plate of a few centimetres diameter and less than a millimetre thick. When such a plate, containing nearly a million channels of 10–15 μm diameter, is placed in front of the fluorescent screen of a single-stage image intensifier tube, brightness gains in excess of 100,000 can easily be obtained. Image tubes of this kind can achieve a better picture quality than cascaded stacks, and lead to smaller and lighter instruments. In addition, channel multiplication has the advantage of local saturation of highlights, owing to the limited supply of secondary electrons available from the wall of each channel. This characteristic substantially reduces the disturbing effect of bright light-sources in the field of view without affecting the gain in darker parts of the scene. The insertion of the channel plate in the paths of the photo-electrons does, however, lead to a loss of some primary electrons which hit the solid web of the plate surrounding each channel, thus missing the entrance to one of the channels.

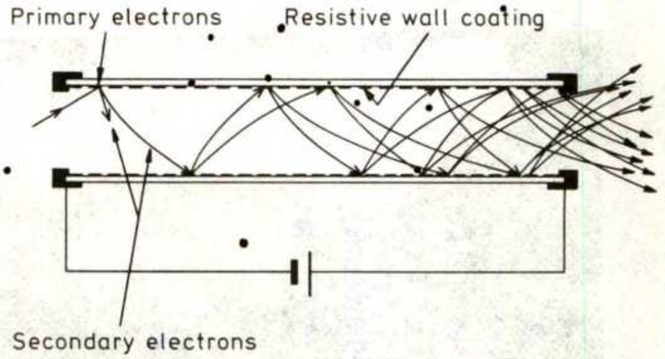


Fig. 6 Illustration of the process of channel electron multiplication.

Channel electron multiplier tubes can be used in small light-weight goggles, as shown in Fig. 7, and are also suitable for other applications. They can, for example, easily be combined with a television camera tube of the vidicon type to provide a comparatively simple approach to low-light-level television[20]. In this case the high-light suppression characteristic of the channel tube, and the ease of providing automatic brightness control are important assets. Figure 8 shows photographs taken from the output screen of a channel tube for two different levels of illumination and brightness gain. The picture taken in starlight shows clearly the scintillations corresponding to individual photons.

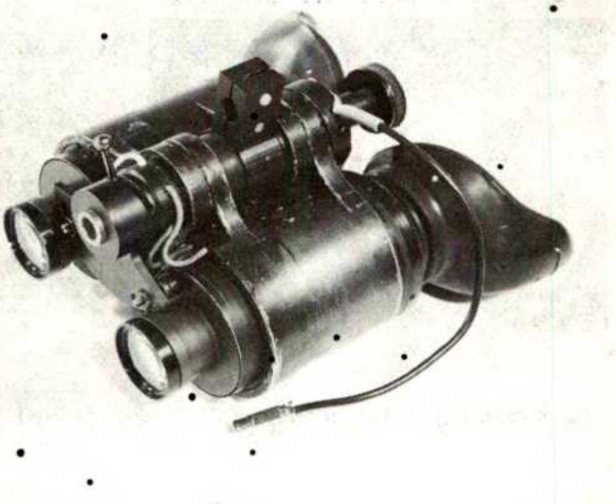
Thermal imaging

An alternative approach to remote sensing of the environment is to use the natural thermal radiation from the scene. The spectral energy distribution of this black-body radiation is given by Planck's law.

For a black body at room temperature this energy peaks at a wavelength of about 10 μm, with very little energy below 2 μm. Figure 2 showed that the photocathodes used in image intensifier tube have practically no sensitivity at wavelengths above 1 μm. It is therefore not possible to convert thermal images to visible wavelengths with the aid of conventional image intensifier tubes, and it is necessary to replace photoemitters as the primary sensors by detectors of which the spectral sensitivity does extend well beyond 1 μm. These can be photoconductive or photovoltaic cells, where absorbed individual infrared photons can create corresponding charge carriers in the semiconductor material. Alternatively a bolometric effect can be used where the absorbed radiation gives rise to local changes in temperature of the detector material, of which a physical parameter with a high temperature coefficient is measured for every picture element.

A black body of 290 K, that is with a temperature close to

Fig. 7 Photograph of a pair of light-weight goggles, containing channel image intensifier tubes.



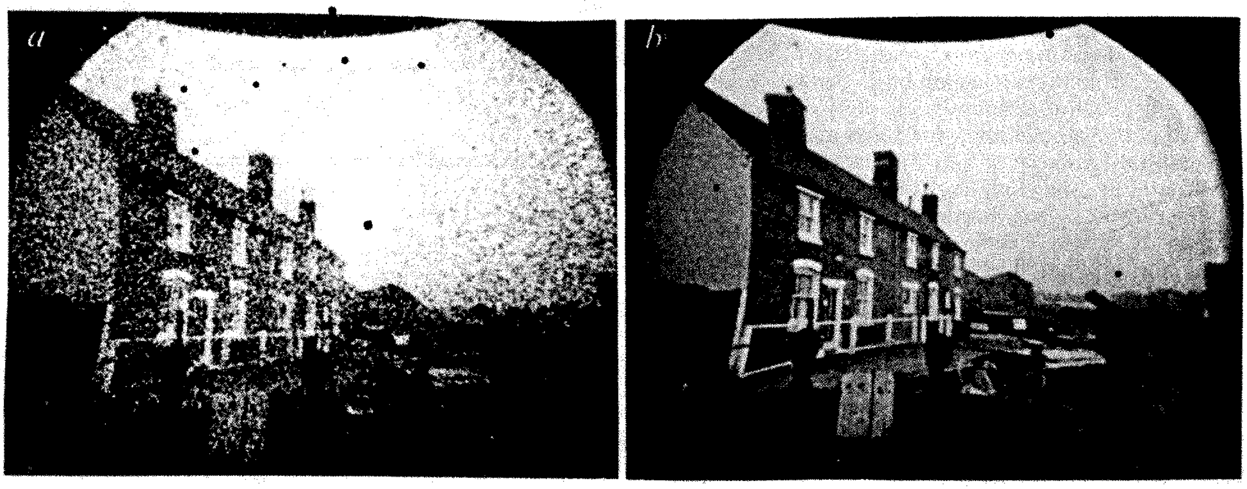


Fig. 8 Photographs taken from the output screen of a channel image intensifier tube, exposure time 1/8 s. *a*, Starlight illumination, brightness gain approximately 100,000. *b*, Full moonlight, brightness gain less than 10,000.

that of most objects in the natural scene, emits approximately 10^{18} photons s^{-1} cm^{-2} in the wavelength region up to 20 μm. (Compare this with the 10^{17} photons s^{-1} cm^{-2} in the visible region incident from the sky in sunlight or 5×10^{9} photons s^{-1} cm^{-2} in moonlight.) The actual numbers of photons available for thermal imaging are thus far larger than those reflected from an object illuminated by the night sky, and even exceed those reflected by sunlit objects by a substantial factor.

In reflected light the image contrast can be very high, exceeding 0.99. But, because the apparent black-body temperature of different natural objects in the nocturnal scene normally differs by not more than about 1 °C, the contrast in the thermal image will be very much lower, and depends on the wavelength region used. For example, if all the photons up to 3 μm wavelength were used, then the contrast for 1 °C temperature difference would be 0.06. This reduces to 0.03 for wavelengths up to 6 μm, to 0.02 up to 10 μm and 0.015 for wavelengths up to 16 μm.

With such a low contrast, a target composed of a large number of small sensors, arranged in a two-dimensional array, can easily give rise to a serious spatial noise, comparable with a 'dirty window' effect, because of small variations in sensitivity and dark-current of the individual sensors, giving rise to variations in output current comparable to those caused by the thermal signal. The simplest way of avoiding this problem is to use one single sensor cell and scan the image mechanically across it. Spatial variations are thus completely eliminated, but the approach is very uneconomical in its use of the incoming radiation and requires high-speed mechanical scanning.

A compromise solution is to use a linear array of sensor cells and scan the thermal image of the scene mechanically across this array in one or more sweeps by wobbling the optics, to constitute the frame scan. The output of each cell in turn can be sampled at high frequency in order to create the conventional video signal. By limiting the number of sensors to at most a few hundred, it is still possible to select arrays with only small differences in dark current and sensitivity of individual sensors, or even to correct for these.

The performance of such thermal imaging systems, using arrays of n sensors, is again noise limited according to a relationship which can readily be derived from the one calculated by Chiari and Jervis[21]

$$\Delta T_N \alpha = \text{constant} \qquad (2)$$

where ΔT_N is the noise equivalent temperature difference, that is, the difference in black-body temperature between two object elements in the scene for which the corresponding signal component of the detector output current just equals the combined noise current of all the cells and α is the angular resolution (in radians). The constant describes system characteristics and can be written as $4(f/D^2)(p/n)^{\frac{1}{2}}(1/M^*)$ where D is once again the diameter of the objective lens (in cm); f its focal length; p the number of cells scanned per second (equivalent to the bandwidth of the video signal) and M^* the figure of merit of the detector material, indicating the sensitivity of the detector to differences of temperature in the scene. The figure of merit also takes into account a given atmospheric absorption of the infrared radiation.

Table 1 gives typical values of M^* for a number of detector

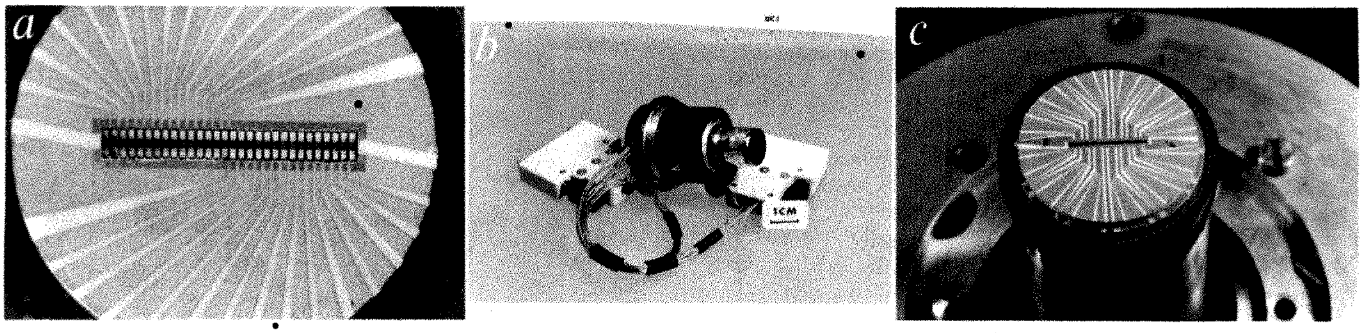


Fig. 9 Photographs of linear arrays of CMT cells, courtesy F. D. Morten of Mullard Limited, Southampton. *a*, 30-element array of CMT cells, 115 × 125 μm. *b*, As (*a*) mounted. (*c*) 192-element array of CMT cells, 50 × 50 μm.

systems produced in arrays, allowing for typical transmission at a range of 2 km (ref. 22). For the smaller values of α the just perceptible temperature differences in the scene, given by equation (2), will again be restricted by the degradation in resolution caused by the optical components. Equation (2) does, however, indicate that high-performance thermal imaging is feasible with linear arrays of sensor cells. Recognisable images of a natural scene can be formed when temperature differences do not exceed about 1 °C. It should, for example, be possible to obtain television-type pictures with a temperature resolution of 0.1 °C, together with an angular resolution of 0.2 mrad and a total viewing angle of about 7°, for an objective lens with $f = 30$ cm and $D = 20$ cm, a picture of 400×600 picture elements and 25 frames per second, if a value for $M^*n^{\frac{1}{2}}$ of about 3.67×10^7 is achieved. This should be obtainable with an array of approximately 150 photoconductive cadmium mercury telluride (CMT) cells ($M^* \sim 3 \times 10^6$) at 77 K, provided that the optics are not yet limiting the resolution.

Sensors of this kind can now be manufactured in arrays of up to several hundred individual elements with sizes typically down to about 50 µm. Arrays of 30 and 192 CMT sensor elements, with leads connecting bonding pads at the edge of the carrier directly to the elements, are illustrated in Figure 9.

The photographs of Figure 10, courtesy of Mr. W. D. Lawson of RSRE, Great Malvern, illustrate the type of thermal picture obtainable in the 8–13-µm band with 30-element arrays of CMT, cooled to 77 K. The angular resolution of the equipment used was 0.25 mrad, the field of view 10° × 4° and the frame rate 25 pictures per second. Arrays and individual cells for thermal imaging systems with lower temperature and angular resolution are also commercially available. These include CMT cells for operation at intermediate temperatures of 193 K to 233 K. These temperatures can be achieved with the aid of comparatively small and compact thermo-electric coolers[23].

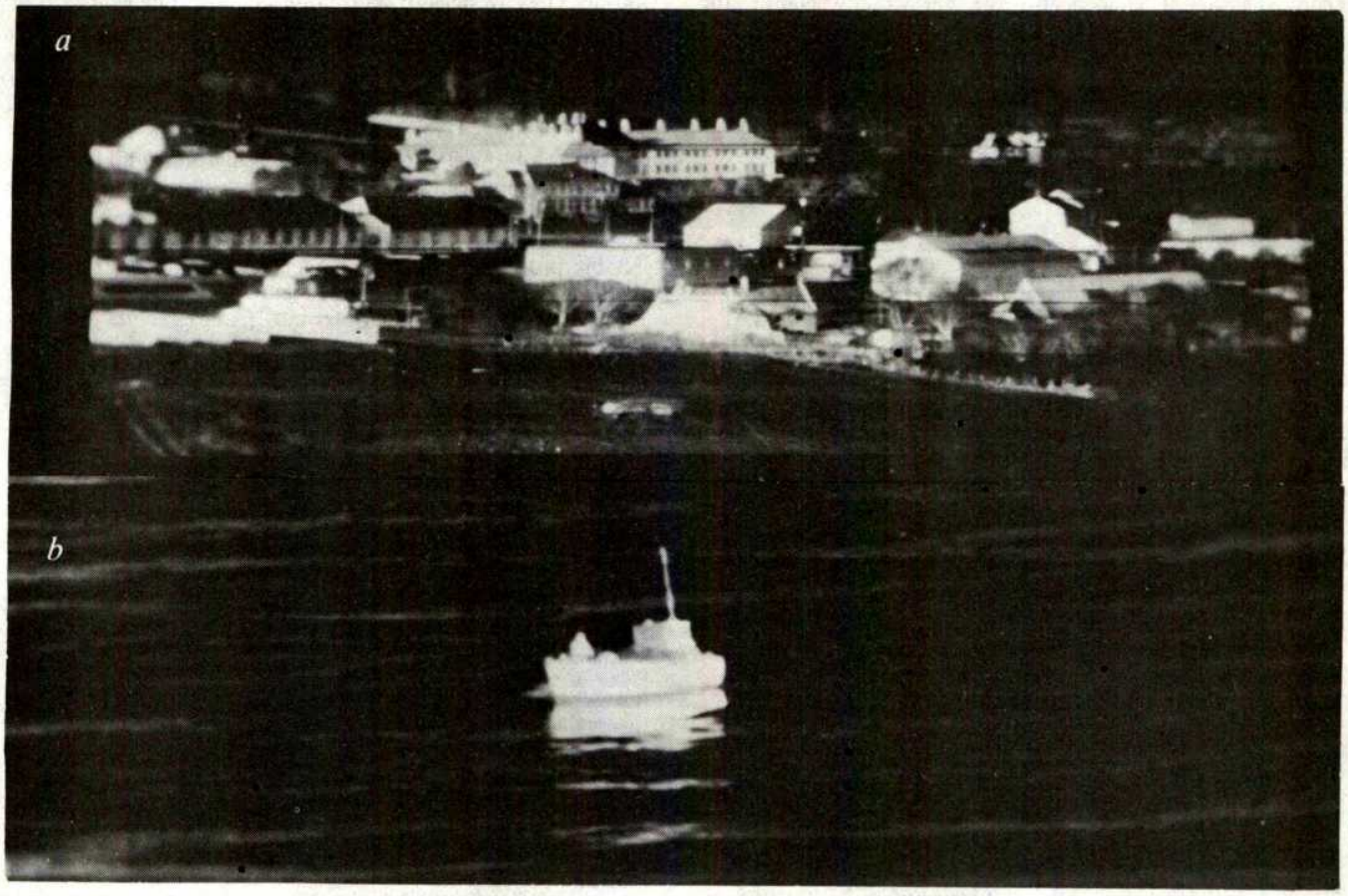


Fig. 10 Thermal pictures obtained with 30-element CMT arrays. *a*, A housing estate in daytime; *b*, A small fishing boat at night.

Table 1 Typical values of M^* for detector systems produced in arrays

Detector systems available	Element temperature	Frequency (Hz)	M^* (cm^{-1}, $\mathrm{Hz}^{\frac{1}{2}}$, K^{-1})
CMT (30 elements)	77 K	5000	4×10^6
CMT (192 elements)	77 K	5000	3×10^6
TGS (128 elements)	295 K	800	10^4
TGS (128 elements)	295 K	125	2×10^4
TGS single element	295 K	800	2×10^4
CMT 3–5 µm band	233 K	5000	10^5
CMT 8–13 µm band	233 K	5000	3×10^4

CMT, cadmium mercury telluride. TGS, triglycine sulphate. M^* is for a 2-km atmospheric transmission path.

A somewhat modified approach to thermal imaging with linear sensor arrays is particularly suitable for airborne photography. In this case an image of the terrain is swept across the sensor or sensors to trace the lines of the picture, whereas the movement of the aircraft provides the much slower continuous scanning motion in the other direction. By recording the video information on a film with continuous uniform movement, a picture of a strip of terrain is gradually built up with high temperature and spatial resolution[24].

An interesting alternative to cooled arrays of photocells is to translate the thermal image into a pattern of electrical charges on a two-dimensional target, and to read these out with the aid of a scanning electron beam, as in a television pick-up tube of the vidicon type, illustrated in Figure 11. Wherever the photoconductive material, which can for example be PbTe (ref. 25) receives infrared radiation, charge can leak to the surface from the positive semi-transparent electrode underneath. The electron beam removes this charge during the next scan, thus creating a signal-current proportional to the local level of illumination.

Instead of using a photoconductive material, it is also possible to make use of the pyroelectric effect[26] exhibited by some materials, such as for instance triglycine sulphate (TGS) or one of its derivatives. Thin slices of such a material acquire electrical charges on the surfaces, whenever the temperature of the material changes. This effect has led to the development of the pyroelectric vidicon[27,28]. Such a tube can translate thermal images of the natural scene into useful video signals with temperature resolutions already down to about 0.2 °C for a 100-line picture.

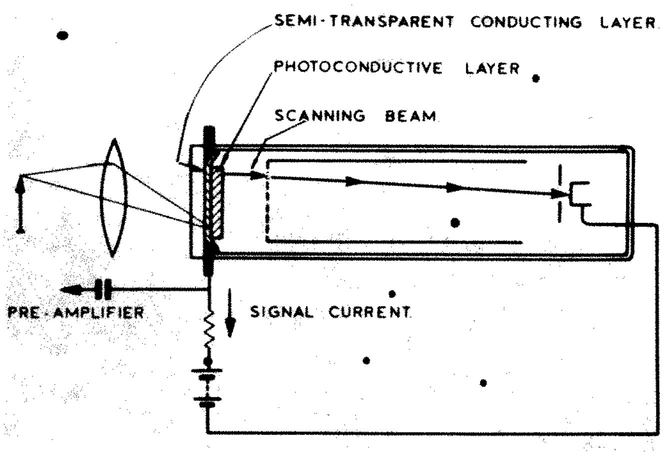


Fig. 11 Schematic diagram of a vidicon.

Further improvements of this approach are still envisaged, both in terms of target design[29,30], and more suitable pyroelectric materials[31,32], which might eventually make it competitive with some of the smaller linear arrays of cooled CMT sensors, but with the advantages of more simplicity, operation at room temperature and not requiring high-speed mechanical scanning.

Whereas image intensification as a means for improved night viewing is now quite close to its theoretical photon-noise limited optimum performance, considerable further improvements in thermal imaging techniques can still be foreseen. These can be expected to result partly from further advances in detector fabrication technology, such as monolithic sensor arrays prepared on chips of single crystal material[33], or even two-dimensional arrays with integral charge-coupled read-out[34–38].

Second, recent advances in signal processing, first developed for improving pictures obtained in the American space programme, can also make a major contribution to increasing the picture quality in thermal imaging, by means of processes like edge enhancement, deblurring, contrast manipulation and extracting small picture signals from noise[39].

Remote sensing methods of this kind, using thermal imaging, are likely to provide a night vision capability to military and security forces[40–43] at longer ranges than can be realised with image intensifiers, partly also because objects of special interest in the scene, like vehicles with their engines running and human bodies, are usually a few tens of degrees hotter than their natural surroundings.

Other applications of thermal imaging

Thermal imaging has so far received its main stimulus as an alternative night viewing aid to image intensifiers for military applications. The thermal pictures received from the scene do, however, provide different information to that normally obtained with reflected visible light, and are therefore also beginning to prove their usefulness in a wide variety of other applications[44–47]. These include geological surveys to map water resources, indicate potential oil and mineral deposits, as well as agricultural inspections to monitor crop development and detect early signs of crop failure due to fungus growth or other diseases.

Other applications are in the fields of medicine[48–50] (thermography), where it may also sometimes be useful to add isothermal contours to the picture[51,52], meteorology, urban planning, energy conservation by optimising the thermal insulation of dwellings, monitoring environmental chemical and thermal pollution, oceanography, astronomy, etc. In some cases the thermal band is divided into a number of separate channels to provide multispectral data[53,54], which can be suitably processed and used to reproduce multicolour pictures in the visible spectrum, thus facilitating even further the identification of particular terrain features with uniquely different spectral characteristics.

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articles

The observability of possible atmospheric removal processes for chlorfluorocarbons 11 and 12

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The existence of tropospheric sinks for CFCs 11 and 12 would have a large impact on projected stratospheric ozone effects. The existence of such sinks, distinct from no sinks, cannot at present be discerned from atmospheric measurements. As presently analysed, tens of years may pass before such a distinction would be possible. This article scrutinises transient mechanisms that could contribute to accomplishing this critical distinction in 10 yr or less.

THE issue of possible stratospheric ozone destruction by halocarbons, and specifically chlorfluorocarbons 11 and 12 (CFCs 11 and 12), depends sensitively upon the presence or absence of tropospheric sinks, as indicated by several theoretical studies[1-7] using steady-state and transient one-dimensional (1-D) parameterised transport calculations. These studies focus on: (1) atmospheric CFC mixing ratios for assumed continuing CFC production; (2) Stratospheric ozone depletion for assumed continuing and halted CFC production.

Notably absent, and the subject of this article, is a direct examination of the information content in transient atmospheric CFC ratios following an assumed abrupt reduction of one or more CFCs.

In analysing relatively weak CFC loss mechanisms, weighed against their production and atmospheric transport processes, the present status of the problem is as follows: photodissociation losses of CFCs 11 and 12 above 30 km are generally established[5]; consensus has not been reached on the existence, or non-existence, of other significant loss mechanisms for CFCs 11 and 12[8-10]; observational data[10,11] are sparse, variable and uncertain[9,11]; 1-D theoretical results are uncertain and globally unresolved [8-10,13,14]; CFC production and release data have been extensively compiled and updated[9,12]; and observational CFC mixing ratio data, sparse at present, are growing with increasing efforts towards absolute calibration and accounting of local background effects[9-11].

Estimates have been made of the potential reductions[8-10] of asymptotic CFC mixing ratios that could occur from other atmospheric loss processes acting in addition to CFC photodissociation. Many decades must pass, however, before asymptotic conditions will occur—even if significant tropospheric removal processes do exist.

This article suggests previously unnoted transient mechanisms and trend analysis that, with appropriate development, could contribute to a resolution of this critical issue of tropospheric losses in 10 yr or less.

Calculation procedure

The purpose of our analysis is to assess the physical observability of tentative tropospheric CFC loss processes, without a detailed consideration of complex chemical mechanisms. Our investigative examples employ a conventional 1-D time-dependent representation of the Earth's atmosphere from 0 to 55 km with arbitrary CFC production and loss processes. Atmospheric transport is represented by the computational artefact of vertical eddy diffusion. The descriptive equations for globally averaged concentrations, $C_i(z,t)$ cm^{-3}, are given by

$$\partial C_i/\partial t = \partial/\partial z\{K(z)\rho[\partial(C_i/\rho)/\partial z]\}+P_i-L_i$$
$$i = \text{CFCs 11 and 12} \qquad (1)$$

The vertical eddy diffusion coefficients are denoted by $K(z)$ and, for illustrative purposes, are assigned the values used by

Fig. 1 Relative tropospheric volume mixing ratios for CF_2Cl_2 (CFC 12) ——●——, no sink; — —○— —, 10-yr tropospheric sink. *a*, Source constant after 1974; *b*, source = 0 after 1974.

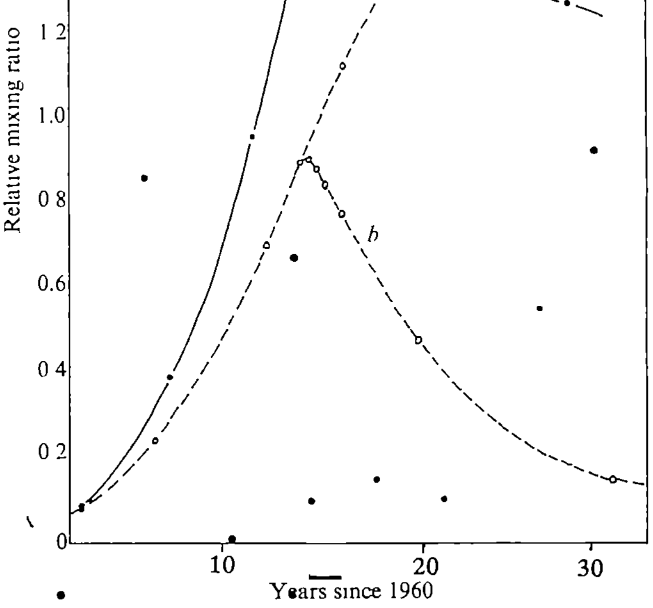


Table 1 CFC mixing ratio trends for the 15–20-yr interval

CFC	Lifetime of assumed tropospheric sink (yr)	Trends for source constant after 1974	Trend ratio (10 yr)/(∞)	Trends for source = 0 after 1974	Trend ratio (10 yr)/(∞)
$CFCl_3$	∞	0.1	~½	−0.02	~2
	10	0.06		−0.04	
CF_2Cl_2	∞	0.2	~½	−0.01	6–7
	10	0.08		−0.07	

Chang[13]. Comparative results with other sets of $K(z)$ appear in refs 9, 15. Natural and anthropogenic CFC production[9] and loss terms are denoted by P_i and L_i, respectively. The atmospheric number density, ρ, is taken from the US Standard Atmosphere[16], and atmospheric mixing ratios are

$$\mu_i(z,t) = C_i(z,t)/\rho(z,t), \quad i = \text{CFCs 11 and 12} \qquad (2)$$

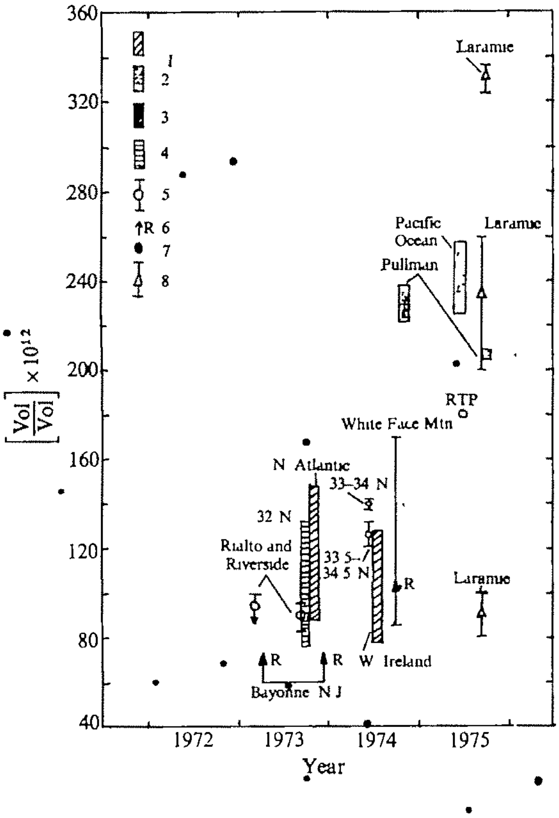
Loss rates are expressed as

$$L_i = J_i\, C_i + (1/\tau_i)\, C_i, \quad i = \text{CFCs 11 and 12} \qquad (3)$$

The photodissociation rates, J_i, are taken from Rowland and Molina[5]. The following additional CFC loss processes will be examined: (1) 10-yr tropospheric sink (τ_i = 10 yr for 0–15 km); (2) 40-yr tropospheric sink (τ_i = 40 yr for 0–15 km); (3) 10-yr stratospheric aerosol sink (τ_i = 10 yr for 15–20 km).

Our calculational procedure was first applied to parameterised transport models with arbitrary sources and sinks by MacCracken and Gelinas[17–19], and extended to include complex photochemical mechanisms by Chang *et al.*[20] This method of casting reactive transport equations into ordinary rate equations for fully implicit, simultaneous integration in space and time is extensively documented, with supportive motivations and assumptions[17–13]. Vertical mesh spacings of 1 km were used, and time-dependent release rates[9] were converted to corresponding globally averaged, time-varying source rates in the 0–1 km zone. Upper boundary conditions of both zero net flux and zero re-entrant flux at 55 km yield the stated results.

Fig. 2 Observed tropospheric mixing ratios for CF_2Cl_2 (CFC 12). Sources: 1, Lovelock; 2, Washington State University; 3, Heidt *et al.*; 4, Naval Research Lab.; 5, University of California, Riverside; 6, Rutgers University; 7, EPA; 8, Goldan *et al.*



Results

Cases (1)–(3) focus directly on circumstances where tropospheric CFC removal processes would be most immediately observable, that is, with inconsequential CFC production, relative to losses. Either abrupt source reductions or total curtailment of CFC 11 and/or 12 can be studied. Total source curtailments are used in our examples because the information content of governing mechanisms is most readily apparent in that limit.

Our primary results are developed from Fig. 1, which presents the relative mixing ratios of infinite against 10-yr, tropospheric lifetimes for CFC 12 with: source rates continuing constant at 1974 levels, subsequent to 1974; or production assumed halted subsequent to 1974. Also calculated, but not plotted, are CFC 11 results which have the same qualitative structure as CFC 12 results. Quantitative differences are noted in Table 1. Also, results for a 40-yr tropospheric sink and a 10-yr stratospheric aerosol sink were calculated, and lie between the 10-yr and infinite lifetime results.

Our analysis now concentrates on relative trends in an effort to minimise the role of absolute uncertainties and poor global resolution in observations and calculations. The following points can be noted: (1) Following curtailments linear trends are immediately established and persist for nearly a decade. (2) The tropospheric trend ratio (Table 1) for curtailed CFC 12 is distinctly different (6–7 against ~2) from that for the other cases, which may present observational and interpretive opportunities for distinguishing any significant tropospheric sinks. (3) Tropospheric trend significance of ±10% may be necessary to distinguish significant tropospheric sinks.

Figures 2 and 3 present existing observational data that seem most representative of background CFC levels[9,11]. We present these data primarily to view trend significance, which is no better than ±(25–50%), even accounting for nothern–southern hemispheric decrements[10]. In fact, because this existing trend data, as well as absolute CFC mixing ratio magnitudes, have sufficient scatter, it is possible that CFC production and release is greater than currently reported and estimated[9,10].

Conclusions

Our investigation was prompted by the prospect of continued indeterminacy of critically significant tropospheric CFC sinks from absolute analyses, and the practical need to make the best use of somewhat randomly collected, sparse and often uncalibrated observational data, coupled with available 1-D theoretical interpretations that incorporate uncertain transport parameterisations with no horizontal resolution. We found, following a source curtailment, that the trend of tropospheric CFC 12 mixing ratios is different by nearly an order of magnitude for 10-yr trophospheric sinks, against no tropospheric sinks. Furthermore, these calculated trends are distinct almost immediately following a source curtailment. Considerations for reducing this mechanism to practical application suggest a number of related points: (1) Concentration on tropospheric measurements may suffice for determining critical CFC sinks, irrespective of atmospheric chemistry details. (2) Tropospheric measurements at permanent, stationary stations would be useful for trend analyses, preferably at several northern and southern

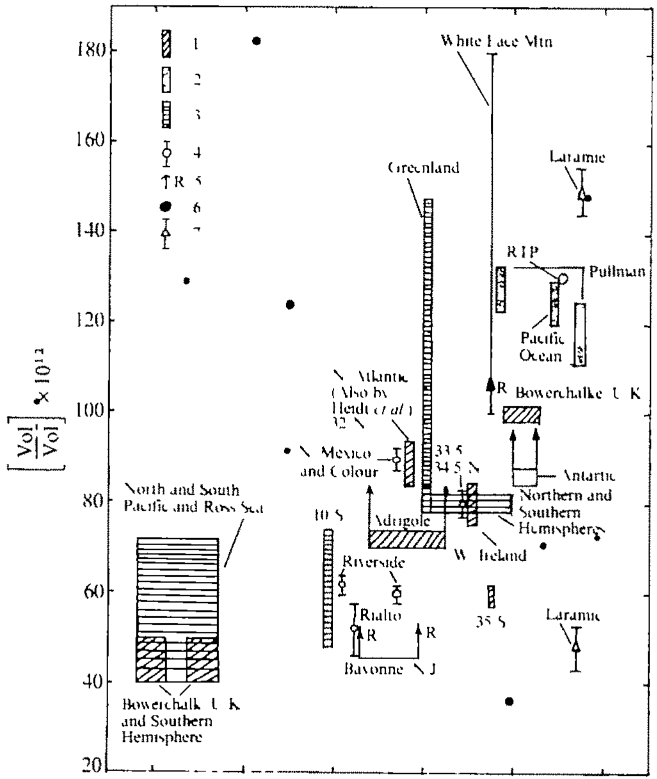


Fig. 3 Observed tropospheric mixing ratios for $CFCl_3$ (CFC 11) Sources 1–7, as in Fig. 2 legend.

latitudes, with many observations taken daily. Siting should maximise the probability of measuring CFC background levels. (3) Observations at each station should include selected tropospheric altitudes, and an array (north, south, east and west) of horizontal sites which may provide a needed measure of local effects. (4) Meaningful trends, inclusive of seasonal variations, could be evaluated station by station, and further processed for both latitudinal and global significance. (5) Absolute calibration for CFC measurements should be established. (6) Natural variabilities in trend data could prove to be too large for 1-D analysis to apply with adequate sensitivity for an explicit determination of possible tropospheric FC sinks. If so, this realisation would be of greatest value earlier, rather than later.

Since this avenue of dynamic analysis is only now put forth for CFCs, a substantial amount of possible future investigation comes immediately to mind. More incisive considerations of CFC data significance and interpretability may be accomplished by analysis of local, meridional and hemispheric trends, in association with global evaluations. These considerations may extend to further analysis of dynamic responses to effective eddy diffusivity variations in 1-D and 2-D, to general atmospheric circulation mechanisms, and to natural radiative variations[24,25].

Several circumstances of gradual against abrupt changes in CFC production, release or substitutions remain for extended investigstion. Correlated and anticorrelated trends of possible CFC substitutions could offer very effective filtering of absolute uncertainties, consistent with the mechanisms presented above.

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Direct demonstration of β-globin mRNA in homozygous Ferrara β_0-thalassaemia patients

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In cases of β_0 thalassaemia from Ferrara, Italy, the β-globin gene is transcribed into mRNA but no protein is synthesised. For these cases there is no hybridisation data suggesting a globin gene structural mutation. This again demonstrates the diverse molecular events which may cause this prevalent hereditary disease.

β-THALASSAEMIA is an hereditary anaemia in which there is an excess of α-globin over β-globin chain synthesis. In homozygotes for β_0-thalassaemia no β-globin is synthesised, and active haemoglobin is only obtained by the compensatory synthesis of δ- and γ-globin chains. The wide geographical distribution and varying clinical severity of β-thalassaemia indicates the existence of several different lesions at the molecular level, each of which could give rise to the reduced synthesis of β-globin chains[1].

Several cases of β_0-thalassaemia have been described for which there is an absence or reduction in β-globin messenger RNA (mRNA$_\beta$) (refs 2–6), as judged by protein synthesis in a cell-free system or by DNA–RNA hybridisation. Cases from Ferrara, Italy, seem to differ from this more usual pattern, in that it has been reported that β-globin chain synthesis can be activated in reticulocytes both *in vivo* after transfusion and *in vitro* by the addition of post-ribosomal supernatant from normal reticulocyte lysates[7,8]. But so far it has not been demonstrated conclusively by direct measurements that mRNA$_\beta$ is present in such a patient.

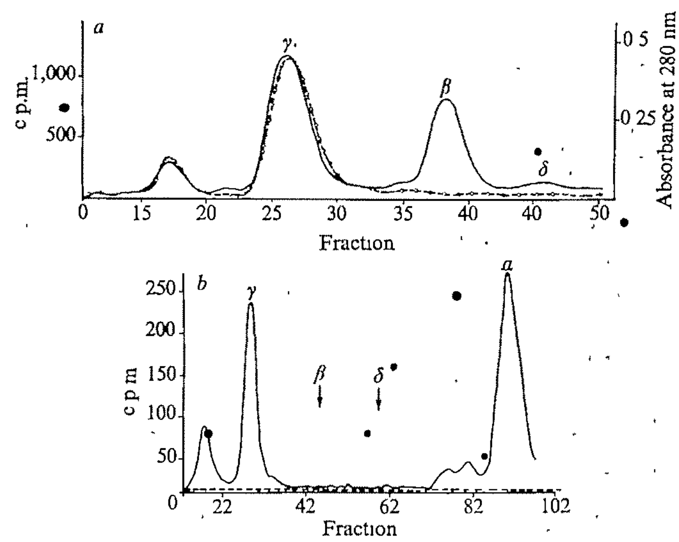

Fig. 1 Globin synthesis in whole cell incubations of reticulocytes from two Ferrara patients. In both cases reticulocytes were incubated with ^{3}H-leucine for 1 h at 37 °C; chain isolation and analysis were carried out on carboxymethylcellulose columns as described previously[8]. Carrier adult globin was added in one case (*a*, patient FR); in the other case (*b*, patient AS) the position of the β- and δ-globin peaks were determined in a parallel run. In both patients, as for the other cases examined, less than 1% of the total amino acid incorporation into globin chromatographs with the β-globin peak. These experiments were performed, in some cases, simultaneously in two laboratories with identical results.

Techniques for preparing complementary DNA (cDNA) probes which can be purified for homology for β-globin genes and mRNA[4] have enabled us to assay directly by hybridisation for mRNA$_\beta$ in several patients with Ferrara thalassaemia both before and after transfusion. Significant levels of mRNA$_\beta$ (up to 12%) have been detected in circulating reticulocytes of patients for whom globin chromatography demonstrates the absence of β-globin chain synthesis. The level of β-globin-specific mRNA sequences in nuclear RNA from the one patient studied is higher still, between 20 and 30%. Ferrara thalassaemia thus differs in molecular pathology from many southern Italian and Asian β_0-thalassaemias, for which the β-globin gene is present but no detectable mRNA is found in the reticulocyte[5], and from South-east Asian α_0-thalassaemia, which is caused by an α-globin gene deletion[9,10]. In other cases of β_0-thalassaemia mRNA$_\beta$ is present, but cannot be translated[23,27].

The conclusions drawn from the DNA–RNA hybridisation experiments will depend on the total absence of β-globin chain synthesis in the cases studied. In each case the total haemoglobin prepared after red-cell lysis was chromatographed on Amberlite and haemoglobin F was eluted[11]. The remaining haemoglobins A and A$_2$ were eluted separately and estimated by absorbance. The sensitivity of this technique allows estimation of as little as 0.2% haemoglobin A in the presence of haemoglobins F and A$_2$. In several cases studied the circulating red blood cells were incubated with ^{3}H-leucine as previously described[7] and the globin chains were extracted and separated on carboxymethylcellulose[12]. This technique is less sensitive but makes possible a direct estimate of β-globin synthesis; as little as approximately 1% β-globin chain can be detected.

In the cases described here there was no haemoglobin A detected on Amberlite chromatography (AS, FR, FB, SM, AM, GC) or on paper electrophoresis (PS) before transfusion. Globin chain chromatography also demonstrated the absence of β-globin chain synthesis (Fig. 1). Therefore it can be concluded, in agreement with our previous findings, that by normal rigorous criteria these patients have a β_0-thalassaemia[13]; γ- and δ-globin synthesis occurs but no β-globin chains are made.

cDNA was prepared as previously described using human globin mRNA as template, oligo(dT) as primer, avian myeloblastosis virus RNA-dependent DNA polymerase as

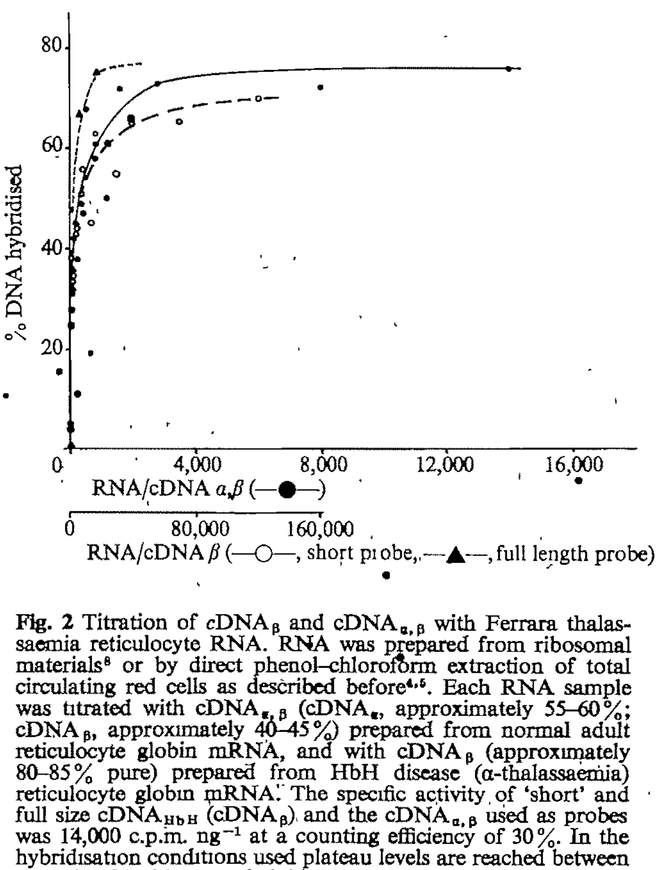

Fig. 2 Titration of cDNA$_\beta$ and cDNA$_{\alpha,\beta}$ with Ferrara thalassaemia reticulocyte RNA. RNA was prepared from ribosomal materials[8] or by direct phenol–chloroform extraction of total circulating red cells as described before[4,6]. Each RNA sample was titrated with cDNA$_{\alpha,\beta}$ (cDNA$_\alpha$, approximately 55–60%; cDNA$_\beta$, approximately 40–45%) prepared from normal adult reticulocyte globin mRNA, and with cDNA$_\beta$ (approximately 80–85% pure) prepared from HbH disease (α-thalassaemia) reticulocyte globin mRNA. The specific activity of 'short' and full size cDNA$_{\text{HbH}}$ (cDNA$_\beta$) and the cDNA$_{\alpha,\beta}$ used as probes was 14,000 c.p.m. ng^{-1} at a counting efficiency of 30%. In the hybridisation conditions used plateau levels are reached between 70 and 80% with normal globin 9S RNA. A constant amount of cDNA$_{\alpha,\beta}$ or cDNA$_\beta$ (18–35 pg) was hybridised with increasing amounts of Ferrara β_0 reticulocyte cytoplasmic RNA, in a volume of 1–2 µl of 50% formamide, 0.5 M NaCl, 25 mM HEPES buffer, pH 6.8, 1 mM EDTA, containing *E. coli* RNA (2.5 mg ml^{-1}). Samples were sealed in silicone-coated glass capilliaries, and incubated at 43 °C for 12–14 d. The percentage of cDNA hybridised was determined by assay with single-strand-specific S1 nuclease, followed by perchloric acid precipitation of hybridised material. Further details are given in previous publications[4,5,8,15]. The ratio mRNA$_\beta$: mRNA$_\alpha$ was calculated by adjusting the abscissa until the initial portions of the hybridisation curves were superimposable, and (after adjusting for the amount of cDNA$_\beta$ present in the cDNA$_{\alpha,\beta}$) taking the ratio from the magnitude of this adjustment. With normal adult globin mRNA the mRNA$_\beta$:mRNA$_\alpha$ ratio obtained by this technique is approximately 0.65. In this case (MA) the ratio mRNA$_\beta$:mRNA$_\alpha$ is 0.05–0.07. Calculations made from initial slopes of hybridisation gave similar values. When the amount of β-mRNA in a preparation (relative to α-mRNA) is as low as 1–20% the initial slope of the hybridisation of the cDNA$_{\alpha,\beta}$ essentially reflects the hybridisation of the cDNA$_\alpha$ component.

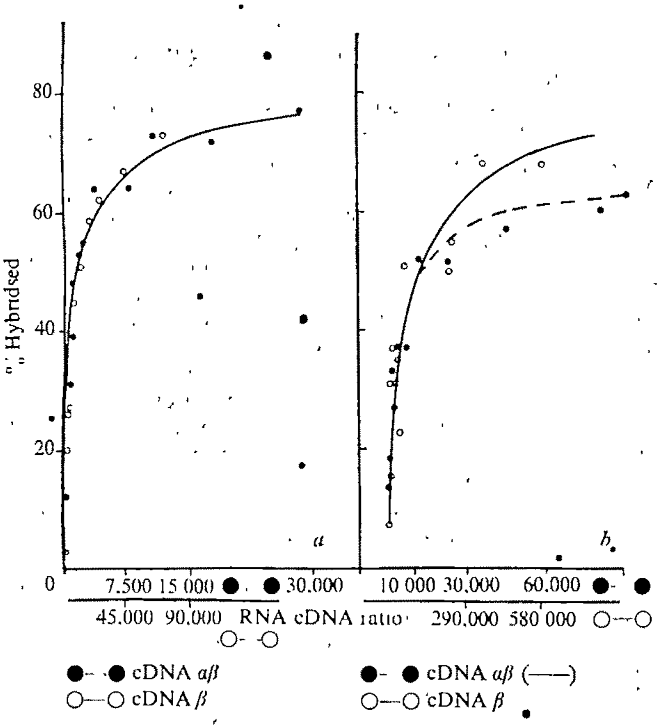

Fig. 3 Titration of bone marrow total nuclear and cytoplasmic RNAs. Cytoplasmic (*a*) and nuclear (*b*) total RNA was prepared from iliac crest bone marrow using a citric acid procedure as described before[15] and titrated with $cDNA_{\alpha,\beta}$ and $cDNA_{\beta}$ (short probe) as described for Fig. 2. In this case (SM) the ratio $cDNA_{\beta}$:$cDNA_{\alpha}$ is 0.10–0.15 for cytoplasmic RNA and 0.20–0.30 for nuclear RNA The ratios required for saturation are much higher for bone marrow than for reticulocytes because of the considerably lower proportion of globin mRNA present[18].

Table 1 $mRNA_{\beta}$ content for different patients

	Patient	%HbA*	%$mRNA_{\beta}$† (in reticulocytes)		
Never transfused					
	AS	<0.2	10–12		
	FR	<0.2	2–3		
	AM	<0.2	2–3 (5–7‡)		
		% β-Globin synthesis	% $mRNA_{\beta}$† (in reticulocytes)	% $mRNA_{\beta}$† in bone marrow cells cytoplasm	nuclei
Transfused					
	PS	31.3	10–20		
	FB§	22.5	12–50		
		14.4	15–20		
	GC	30.8	11–15		
	SM	18.5	—	10–15	20–30

β-Globin synthesis was determined 3–4 weeks after transfusion in intact reticulocyte incubations[6]. The values reported are relative to α-globin synthesis.

* Relative to total haemoglobin.

† Relative to $mRNA_{\alpha}$. In all experiments reported, $cDNA_{HbH}$ hybridisation went essentially to completion (68–80%).

‡ Determined on RNA extracted from ribosomal material[6].

§ Determinations were on blood samples taken after two different transfusions.

enzyme, and ^{3}H-dCTP as the only labelled deoxynucleotide triphosphate[4,5,8,15,16]. For most of the experiments reported, 'short' cDNAs (complementary to the 3′-end half of the mRNA) were prepared; however, in a few instances full size cDNAs were used[17]. Human globin mRNA was obtained from reticulocytes from a subject with a non-thalassaemic haemolytic anaemia ($mRNA_{\alpha,\beta}$) and from a patient with haemoglobin H disease (α thalassaemia), in which the proportion of $mRNA_{\beta}$ is already increased to approximately 80–85% ($mRNA_{\beta}$). These mRNAs were transcribed to give $cDNA_{\alpha,\beta}$ and $cDNA_{HbH}$, which were used directly as probes for $mRNA_{\alpha}$ and $mRNA_{\beta}$ sequences in various RNA preparations from the patients with β_0-thalassaemia (Ferrara). The proportion of $mRNA_{\beta}$ relative to $mRNA_{\alpha}$ was estimated by comparing the initial slopes of the hybridisations with the cDNAs by adjusting the scale of the x axes to make the two hybridisation curves superimposable. The untransfused patients studied (three cases) showed $mRNA_{\beta}$ levels from 2 to 12% in reticulocytes as a proportion of $mRNA_{\alpha}$ (Fig. 2). In four patients $mRNA_{\beta}$ was determined 3–4 weeks after transfusion, when β-globin synthesis was demonstrable in intact reticulocyte incubation. In three of these cases the reticulocyte $mRNA_{\beta}$ levels were between 10 and 20%. In the fourth case a bone marrow sample was examined; the proportion of nuclear RNA sequences hybridising to $cDNA_{\beta}$ was found to be two or three times higher than in cytoplasmic RNA (Fig. 3). The level of $mRNA_{\beta}$ in the circulating reticulocytes of the same patient 5 weeks after transfusion, when β-globin synthesis in intact erythrocytes had become undetectable, was 2%. The results are summarised in Table 1.

The methods for estimating the absolute amounts of $mRNA_{\beta}$ relative to $mRNA_{\alpha}$ are somewhat imprecise, particularly because the calculation depends on the difference between hybridisation to $cDNA_{\beta}$ and to $cDNA_{\alpha,\beta}$ and might give a maximum error of as much as 30% for each value determined. But the fact that the hybridisations go to completion compared with hybridisations to normal reticulocyte RNA for each probe at usual low RNA: cDNA ratios demonstrates the presence of sequences indistinguishable from $mRNA_{\beta}$ by hybridisation criteria in circulating reticulocytes from these cases of β_0-thalassaemia In this respect we wish to stress that, even when a full size $cDNA_{HbH}$, is used, protection of this probe from S_1 nuclease to the same extent as with normal $mRNA_{\alpha,\beta}$ is obtained. Even allowing for errors, the $mRNA_{\beta}$ levels determined are very much higher than the maximum level of β-globin chain synthesis which might have remained undetected, if any, in the untransfused cases (Fig. 2). No $mRNA_{\beta}$ could be detected in identical experiments run in parallel with cytoplasmic RNA from two non-Ferrara cases of β_0-thalassaemia, one of which was examined 3–4 weeks after transfusion[5]; levels of less than 1% of β-mRNA-like sequences have been found consistently in a series of 10 β_0-thalassaemic patients of non-Ferrara origin (P.C., B.G. and S.O., unpublished observations); thus a contribution of $mRNA_\delta$ to hybridisation could not explain the results obtained, as the haemoglobin A_2 levels were similar in all cases.

The thermal stability of the hybrid between Ferrara RNA (from transfused and untransfused patients) and $cDNA_{HbH}$ was 2 °C lower than that of the hybrid between normal 9S mRNA and $cDNA_{HbH}$ (Fig. 4). This confirms that the hybridisation observed is due to $mRNA_\beta$ sequences or sequences very similar to $mRNA_\beta$; the smooth form of the melting curve argues for accurate hybrid formation along the entire sequence. The nucleotide divergence corresponding to a ΔT_m of 2 °C is between 0.5 and 1.0%[18–21]; however, the authors are not sure the difference is significant, as thalassaemic mRNA samples are often degraded owing to difficulties in collection. An effect on melting temperature of this magnitude has been demonstrated for the hybridisation of short rabbit globin mRNA transcripts to cDNA[22]. The melting behaviour of a $cDNA_\beta$–$mRNA_\delta$ hybrid is not yet known.

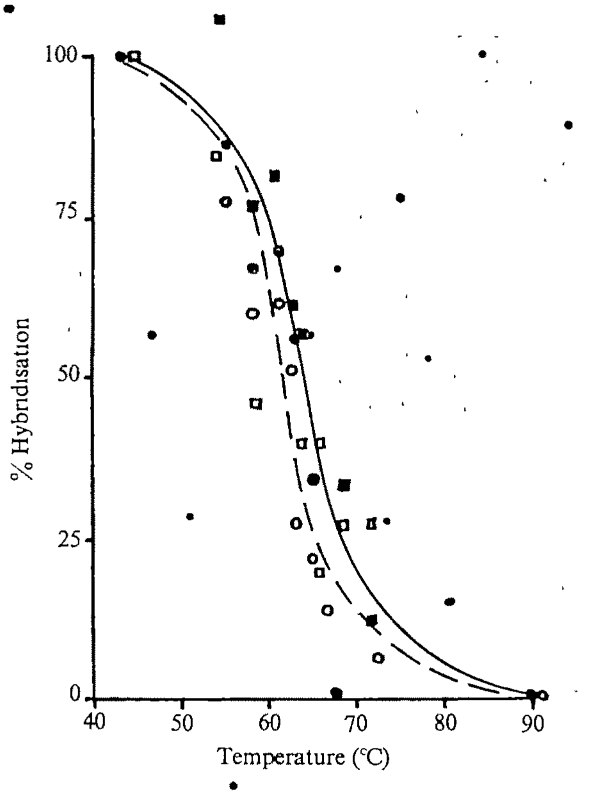

Fig. 4 Melting profiles of normal and Ferrara globin mRNA to $cDNA_\beta$. 20 pg 'short' $cDNA_{HbH}$ were hybridised in individual capillaries with 400 pg normal globin 9S RNA or either 0.9 μg total RNA (AM, ribosomal) or 5.6 μg total RNA (GC) in 1 μl hybridisation buffer at 43 °C for 13 d. Both AM and GC were shown to be β_0 thalassaemics by Amberlite chromatography. At the end of the incubation, capillaries were heated to the indicated temperature, quickly cooled to 0 °C, diluted with S1 nuclease assay buffer and frozen until assayed with S1 nuclease. Data are expressed as percentage of maximum hybridisation obtained; background S1 resistance is subtracted. In this experiment $cDNA_{HbH}$ hybridised to 73% with normal 9S mRNA and to 65% with Ferrara thalassaemia RNA ● and ■, Normal RNA; ○, RNA from GC; □, RNA from AM.

Hybridisation to chain-specific cDNA demonstrates the presence of homologous mRNA sequences, and because there is little evidence of cross hybridisation in the globin gene system[4,5], these sequences demonstrated with $cDNA_\beta$ arise from transcription of the β-globin gene. In the cases considered at least some complete β-globin mRNA molecules must be present, for full size $cDNA_{HbH}$ can hybridise to completion to Ferrara mRNA.

Attempts to demonstrate synthesis of β-globin directed by isolated mRNA in a heterologous cell-free system have been disappointing, although Pritchard *et al.*[14], using RNA from patient BS, reported the synthesis of a fraction with the chromatographic characteristics of β-globin chains. Because of insufficient radioactivity in the peptides Pritchard *et al.*[14] were not able to identify the peak which chromatographs with β globin unequivocally by fingerprint analysis. Further experiments to this end will be undertaken when enough Ferrara thalassaemia mRNA becomes available.

In Ferrara thalassaemia, apparently normal $mRNA_\beta$ found in erythroid cells which hybridises to $cDNA_\beta$, can be activated *in vitro* by the addition of normal post-ribosomal supernatant[7] and can be activated *in vivo* by transfusion with normal blood[6]; the latter finding has been confirmed in the patients studied in the work reprinted here. The untranslated β-globin mRNA apparently decays faster than normal, as indicated by the higher level of $mRNA_\beta$ found in bone marrow relative to reticulocytes, and by the lower levels of reticulocyte $mRNA_\beta$ found when there has been no transfusion with respect to post-transfusional conditions, when β-globin synthesis occurs. It has been shown that high levels of untranslated $mRNA_\beta$ are present in β_0-thalassaemia among Americans of Chinese descent[23], while some $\delta\beta_0$-thalassaemias and hereditary persistence of foetal haemoglobin are caused by gene deletion[17,24–26]. There are many cases of β_0-thalassaemia where the gene for β globin is present but $mRNA_\beta$ is absent (ref. 5 and P. Tolstoshev and S.O., unpublished observations).

B. Forget (personal communication) has reported similar findings recently, using RNA extracted from samples of blood from one of the patients described here. Ramirez *et al.*[26] have reported the isolation of mRNA from Ferrara thalassaemic blood which hybridises incompletely with $cDNA_\beta$ and have postulated a structurally abnormal $mRNA_\beta$ which does not hybridise to completion. Our results do not agree with theirs.

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5′-Terminal structure and mRNA stability

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Reovirus mRNAs with 5′-terminal m⁷GpppG^m or GpppG are more stable than mRNA containing unblocked ppG 5′-ends when injected into Xenopus laevis *oocytes or incubated in cell-free protein synthesising extracts of wheat germ and mouse L cells. The greater stability of mRNA with blocked 5′ termini is not dependent upon translation but seems to result from protection against 5′-exonucleolytic degradation.*

Most eukaryotic mRNAs including reovirus mRNA contain a blocked, methylated 5′-terminal 'cap', $m^7G(5')ppp(5')X$ (ref. 1). The presence of this structure is required for efficient translation of reovirus mRNA in a wheat germ protein synthesising extract[2]. It also facilitates the function of other viral and cellular mRNAs in cell-free systems derived from animal and plant cells[3–7]. The cap acts at the level of initiation[1], apparently by promoting the formation of ribonuclease-resistant, stable complexes between mRNA and ribosomes[8,9].

It was of interest to determine whether the stability of mRNA is also affected by its 5′-terminal structure. Several eukaryotic mRNAs were previously found to be more stable after microinjection into *Xenopus laevis* oocytes than ribosomal RNAs[10] which have phosphorylated, unblocked 5′ ends. Capped mRNAs can be 'unblocked' by β-elimination, that is, chemical removal of the 5′-terminal m^7G. Although this procedure decreases the translation of treated mRNA, it also alters the 3′ terminus and possibly internal sites in the RNA chains. In an attempt to avoid these complications in studies on the role of the cap in eukaryotic protein synthesis, we established conditions for the selective *in vitro* synthesis of reovirus mRNA with 5′-termini of three different types: unblocked (ppG), blocked (GpppG) and capped (m^7GpppG^m) (ref. 11). We report here that reovirus mRNAs with blocked or capped 5′ termini are more stable than unblocked or β-eliminated viral mRNAs in *X. laevis* oocytes and protein synthesising extracts of wheat germ and L cells. In reticulocyte lysates, however, the mRNAs are similarly stable. In addition, the results suggest that unblocked mRNA is degraded exonucleolytically from the 5′-end.

Increased stability of reovirus mRNA containing blocked 5′ termini

Radioactive reovirus mRNAs were synthesised in the presence of α-^{32}P-UTP under previously specified conditions[11] designed to obtain molecules with predominately (>93%) one type of 5′-end. In addition to the three types noted above, viral mRNAs with 5′-terminal pppG and $pppG^m$ were prepared by β-elimination of blocked and capped mRNAs, respectively. For stability measurements, ^{32}P-labelled samples were microinjected into *X. laevis* oocytes[10]. At the indicated time intervals, the oocytes were homogenised and the lysates analysed for total and acid-precipitable radioactivity. mRNAs with blocked 5′ termini were apparently more stable than those with unblocked, phosphorylated 5′ ends (Fig. 1). For example, 8 h after injection of mRNAs containing GpppG or m^7GpppG^m, ~55% of the intracellular radioactivity remained acid-precipitable. By contrast, values of 20–25% were obtained for the three mRNA samples with unblocked 5′ termini, that is, ppG, pppG and $pppG^m$.

Radioactive material was re-isolated from oocytes at intervals after injection of ^{32}P-labelled mRNA and analysed by sedimentation velocity centrifugation. At each time the acid-precipitable radioactivity was present mostly in full-length viral mRNA rather than RNA fragments. Sedimentation profiles of the untreated samples obtained before injection into oocytes included the large (l), medium (m), and small (s) size classes of

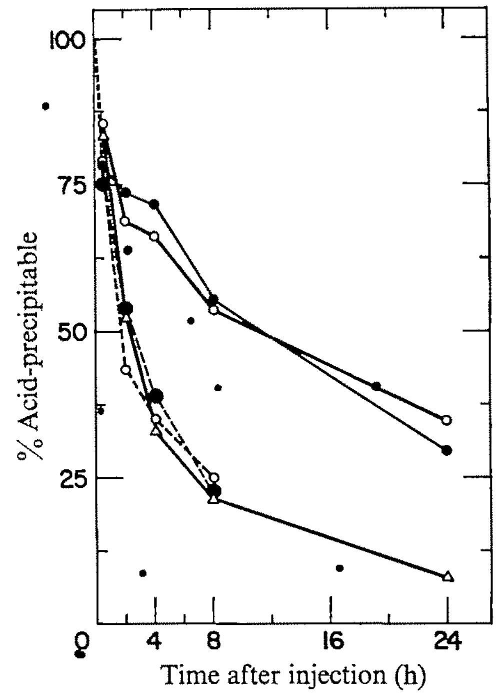

Fig. 1 Degradation of reovirus mRNA injected into *X. laevis* oocytes. Reovirus mRNA was synthesised *in vitro* by the virion-associated RNA polymerase in the presence of α-^{32}P-UTP in conditions[11] that yielded ^{32}P-labelled products (final specific activity = 1.2×10^6 c.p.m. per μg RNA) containing 5′-terminal 94% ppG (with 6% GpppG), 93% GpppG (with 7% ppG), or >94% m^7GpppG^m. Aliquots of the last two samples were oxidised with periodate and treated with aniline to obtain mRNA with 5′-terminal pppG and $pppG^m$ (ref. 21). The β-elimination was >90% complete as monitored with ^{3}H-methyl-labelled mRNA. RNAs were purified by Sephadex G-100 gel filtration, passed through sterile 0.22-μm filters, reprecipitated with ethanol and dissolved in H_2O at concentrations of 3.3 μg μl^{-1}. For each time point, 10 oocytes were injected with 50 nl of RNA solution per oocyte and incubated at room temperature in 5 ml of amphibian media consisting of 15 mM Tris buffer *p*H 7.4, 88 mM NaCl, 1.3 mM KCl, 0.7 mM $CaCl_2$ and 0.8 mM $MgCl_2$ as suggested by Dr L. Burzio, Rockefeller University. At the indicated times, oocytes were removed, washed three times with 2 ml of media, resuspended in 0.2 ml of 50 mM Tris-HCl, *p*H 7.6 containing 0.1 M NaCl, 5 mM EDTA, and 0.5% sodium dodecyl sulphate and homogenised in a Dounce homogeniser. The lysates were extracted with an equal volume of redistilled, H_2O-saturated phenol, centrifuged (3000 r.p.m., 10 min), and the aqueous layers were assayed for total and acid-precipitable radioactivity by counting aliquots directly in Aquasol and on Millipore filters after precipitation in 5% trichloroacetic acid. Because the recovery of intracellular radioactivity varied (23–79% of the estimated amount of micro-injected mRNA) presumably due to leakage, values were normalised by expressing them as a percentage of recovered c.p.m. that were acid-precipitable. mRNAs with 5′-terminal m^7GpppG^m (●), GpppG (○), ppG (△), $pppG^m$ (●--●) and pppG (○--○).

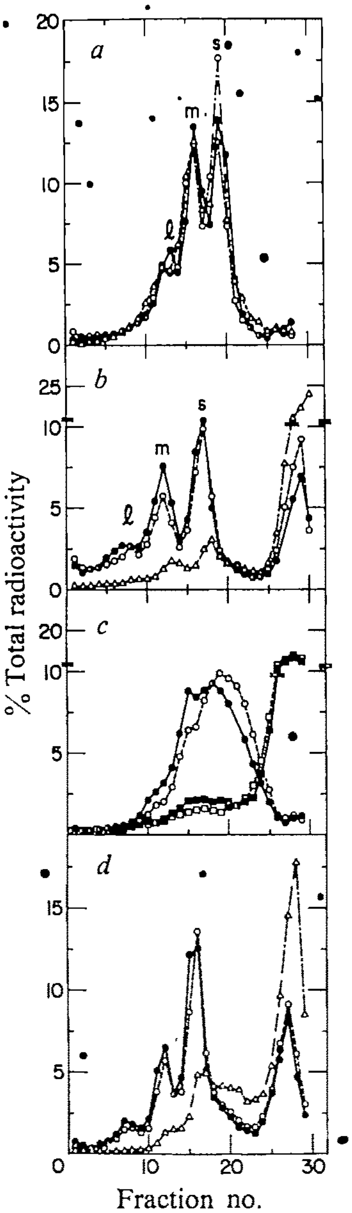

Fig. 2 Sedimentation analysis of reovirus mRNA before and after incubation in oocytes or wheat germ extract. *a*, mRNA synthesised in the presence of α-^{32}P-UTP was purified by gel filtration and sedimented in 5–30% glycerol gradients (20 mM Tris-HCl, *p*H 7.6, 0.1 M NaCl, 1 mM EDTA; SW41–33,000 r.p.m., 16 h). Fractions of 0.4 ml were collected and counted in Aquasol. *b*, The mRNAs containing different types of 5′-ends were injected into oocytes, reisolated after 8 h and analysed as in (*a*). *c*, Blocked and capped 5′-ends of mRNA were converted to 5′-pppG and pppG^m, respectively, by β-elimination[11]. The treated mRNAs were sedimented before (pppG^m, ●; pppG, ○), and after incubation (pppG^m, ■; pppG □) for 8 h in oocytes. *d*, mRNAs synthesised with α-^{32}P-UTP (1 μg, 46–55 × 10^3 c.p.m.) were incubated in wheat germ extract for 10 min at 26 °C. Radioactivity was then recovered by phenol extraction and analysed as in (*a*). For panels *a*, *b* and *d*, mRNAs with 5′-terminal m^{7}GpppG^m (●); GpppG (○); ppG (△).

reovirus mRNA and no slowly sedimenting material (Fig. 2*a*). Following incubation there was a progressive reduction in the proportion of radioactivity in viral mRNA and a corresponding increase in low molecular weight material which was acid-soluble. At all times, however, the fraction of radioactivity sedimenting as intact mRNA was greater for the samples that contained blocked as compared to unblocked 5′ termini. This can be seen in the sedimentation patterns of samples extracted 8 h after injection (Fig. 2*b*). Sixty per cent of the radioactivity in the mRNAs with 5′-terminal m^{7}GpppG^m or GpppG sedimented in the l, m and s positions while less than 20% of the mRNA containing 5′-ppG remained undegraded. Similar results were obtained with β-eliminated mRNAs containing pppG^m and pppG at the 5′-ends (Fig. 2*c*). Although the chemically-treated RNAs were not resolved into the three size classes, possibly because some chains were nicked during β-elimination, the preparations before injection contained very little radioactivity sedimenting at the top of the gradient. By 8 h after injection, more than 75% of both RNAs had been degraded to slowly sedimenting material. Since mRNAs terminating in pppG^m and ppG showed similar rates and patterns of degradation (Figs 1, 2*b* and *c*), an increase in the number of 5′-phosphates and 2′-*O*-methylation of the 5′-terminal residue did not stabilise the mRNA.

Degradation of the various mRNAs in each case resulted in low molecular weight products and no skewing of the mRNA peaks, suggesting exo- rather than endonucleolytic cleavage. This effect can be seen most clearly with mRNA that had not been chemically treated (Fig. 2*b*). Allende *et al.*[10] also observed that the stable fraction of *Dictyostelium* mRNA extracted from *Xenopus* oocytes 24 h after injection cosedimented with uninjected marker mRNA. Our findings that the 5′-unblocked

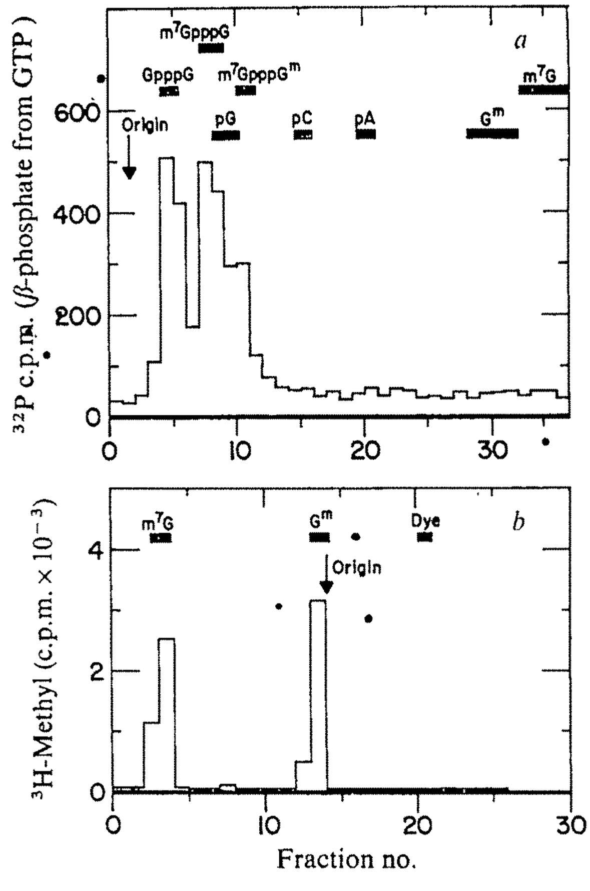

Fig. 3 Analysis of 5′-terminally labelled mRNA after incubation in oocytes. *a*, Reovirus mRNA was synthesised *in vitro* in the presence of β,γ-^{32}P-GTP, *S*-adenosylhomocysteine and inorganic pyrophosphatase to obtain products with 5′-terminal Gpp*pG (ref. 11). The purified mRNA was microinjected (a total of 1 μg and 16,000 c.p.m. into 50 oocytes), incubated for 9 h, re-extracted, digested with P$_1$ nuclease and alkaline phosphatase and analysed by paper chromatography in isobutyric acid—0.5 M NH$_4$OH (10/6 v/v)[12]. *b*, ^{3}H-methyl-labelled mRNA containing 5′-terminal m^{7}GpppG^m was injected (50 oocytes each with 50 nl, 0.08 μg, 1,200 c.p.m.) and after 24 h was re-isolated, digested with P$_1$ nuclease and analysed by high voltage paper electrophoresis at *p*H 3.5 (ref 12). The enzyme-resistant, ^{3}H-labelled blocked structures migrating as a peak close to pG were eluted, digested with nucleotide pyrophosphatase and phosphatase and re-analysed by electrophoresis (60 V cm^{-1}, 45 min). Marker compounds were located under ultraviolet light, and 1-cm strips were cut and counted in toluene-based scintillant[12].

mRNAs are less stable than blocked mRNAs, together with the earlier report[10], suggest that mRNA degradation in oocytes occurs from the 5′-end by an exonucleolytic, processive mechanism.

Studies of the fate of eukaryotic mRNAs in *X. laevis* oocytes showed that puromycin treatment decreased stability, and it was proposed that association with ribosomes affords some protection of mRNA against nucleolytic degradation[10]. Because methylation of reovirus mRNA promotes initiation complex formation *in vitro*[1–8], we tested whether the 5′-terminal GpppG in injected viral mRNA was converted to m^{7}GpppG$^{(m)}$ by methyl transferase(s) in oocytes. mRNA was synthesised with β-γ-^{32}P-GTP as radioactive precursor in order to label specifically the blocked 5′-terminal structure, Gpp*pG (refs 11, 12). In the synthetic conditions used[11], the mRNA contained 5′-Gpp*pG (93%) and small (7%) amounts of 5′-p*pG. The RNA was injected into oocytes, recovered after 9 h and analysed as described in the legend to Fig. 3 to identify the 5′-terminal structure. About 40% of the ^{32}P-labelled, blocked 5′-termini remained unmethylated and migrated in the position of GpppG (Fig. 3*a*). The rest of the Gpp*pG 5′-ends were methylated, however, and enzyme treatment of the mRNA yielded a mixture of m^{7}Gpp*pG and m^{7}Gpp*pG^m. We conclude that *X. laevis* oocytes, like protein synthesising extracts from plant and animal cells[2,4–7], contain enzyme activities that methylate the 5′-end of reovirus mRNA and presumably other mRNAs as well. Because the blocked reovirus mRNA in oocytes was only partially methylated at the 5′-end (~60% conversion of GpppG to m^{7}GpppG$^{(m)}$ at 9 hr), this event cannot account for stability since the degradation kinetics of unmethylated, blocked mRNA and capped mRNA are almost identical during the 24 h after microinjection and both mRNAs are equally more stable than unblocked mRNA (Fig. 1). Consistent with this conclusion, blocked mRNA was also more stable than unblocked mRNA in wheat germ protein synthesising extracts in conditions that prevented methylation.

In order to determine if caps in mRNA turn over, reovirus mRNA synthesised in the presence of ^{3}H-methyl-*S*-adenosylmethionine and labelled exclusively in the 5′-terminal m^{7}GpppG^m was injected into oocytes. After 24 h, the RNA was reisolated, digested with P$_1$ nuclease and phosphatase, and analysed by paper electrophoresis[12]. Over 90% of the radioactivity migrated in the position of blocked structures (data not shown). This material was eluted, digested with nucleotide pyrophosphatase and alkaline phosphatase, and re-analysed by electrophoresis (Fig. 3*b*). Radioactivity was equally distributed between two ^{3}H-methyl-labelled components migrating in the positions of 7-methylguanosine (m^{7}G = 3,680 c.p.m.) and 2′-*O*-methylguanosine (G^m = 3,690 c.p.m.). Thus, both methylated residues in caps were conserved and the 5′-terminal m^{7}G did not turn over following injection of reovirus mRNA into *X. laevis* oocytes. Although an enzyme activity that cleaves m^{7}G-containing cap structures has been detected in animal cell extracts, it did not hydrolyse caps in intact mRNA[13]. A novel phosphodiesterase that cleaves pyrophosphate linkages in mRNA has been purified from tobacco cells[14]. In our studies, however, there was no evidence for differential turnover of caps when reovirus ^{3}H-methyl-labelled mRNA was incubated for 90 min in conditions of protein synthesis in wheat germ extract and then analysed as in Fig. 3*b* (unpublished results). As reported for cellular mRNAs in mouse kidney[15] and L cells[16], caps in reovirus mRNA apparently decay with the mRNA chain when incubated in *X. laevis* oocytes or wheat germ extract.

Reovirus mRNA degradation independent of translation in wheat germ extract

Reovirus mRNAs with blocked or capped 5′-termini are more stable than unblocked mRNAs in oocytes, consistent with 5′-exonucleolytic degradation. Although *X. laevis* oocytes are useful for *in vivo* studies on mRNA stability and function[10,17], it is not yet known if reovirus mRNAs are translated in this system. To investigate the relationship between translation and the stabilities of mRNAs with variable 5′-terminal structures, studies were performed using wheat germ protein synthesising extract which was shown previously to translate reovirus mRNA containing 5′-terminal m^{7}GpppG^m (ref. 2). The same viral mRNA with 5′-terminal GpppG or ppG functions poorly or not at all in protein synthesis when the conversion of GpppG ends to m^{7}GpppG by wheat germ endogenous methyl transferase(s) is prevented by addition of *S*-adenosylhomocysteine[2]. While the presence of this methylation inhibitor blocks translation of unmethylated reovirus mRNA in wheat germ extract, it does not affect protein synthesis directed by mRNA that is pre-methylated during transcription. As found before, and shown in Fig. 4*a*, only capped mRNA was efficiently translated in the presence of *S*-adenosylhomocysteine. Under the same conditions, the relative mRNA stabilities in wheat germ extract were similar to those found with microinjected oocytes, that is, the capped and blocked mRNAs were both more stable than unblocked mRNA (Fig. 4*b*). Since capped mRNA (that is, blocked and methylated) forms stable complexes with wheat germ ribosomes while blocked but unmethylated mRNA does not[8], the similar stability observed for these two types of mRNAs is not due to protection by association with ribosomes. Thus, increased stability seems to be an intrinsic property of mRNAs with blocked 5′-terminal structures. From the results shown in Figs 4*a* and *b*, it seems that 5′-blocking without methylation is sufficient to enhance reovirus

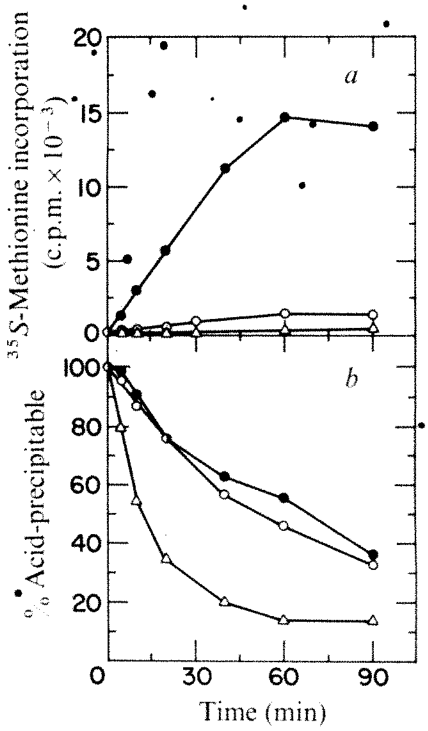

Fig. 4 Translation and degradation of reovirus mRNAs in wheat germ extract in the presence of the methylation inhibitor, *S*-adenosylhomocysteine (Adohcy). *a*, For protein synthesis the incubation mixture (50 µl) contained 12 mM HEPES buffer (*p*H 7.3), 3 mM Mg acetate, 1 mM ATP, 0.05 mM GTP, 5 mM creatine phosphate, 10 µg creatine phosphate kinase, 75 mM KCl, 2 mM dithiothreitol, 100 µM Adohcy, ^{35}S-methionine (10 µCi, 250 Ci mol^{-1}), 0.01 mM of each of the other 19 amino acids, 1 µg reovirus mRNA, and wheat germ extract equivalent to 215 µg protein prepared as before[2]. Incubation was at 26 °C, and 5 µl aliquots were collected in 0.2 ml 5% trichloroacetic acid, heated for 10 min at 90 °C, and the acid-precipitable radioactivity collected on Millipore filters was counted in toluene-based scintillant. *b*, Degradation was measured in the same reaction conditions, except that ^{35}S-methionine was replaced by 0.01 mM non-radioactive methionine and ^{21}P- labelled reovirus mRNAs (1 µg, 110–130 × 10^3 c.p.m.) prepared with α-^{23}P-UTP were used. Incubation was at 26 °C; 5-µl aliquots were extracted and radioactivity precipitated in 5% trichloroacetic acid was measured as described in (*a*). mRNAs contained 5′-terminal m^{7}GpppG^m (●), GpppG (○) and ppG (△).

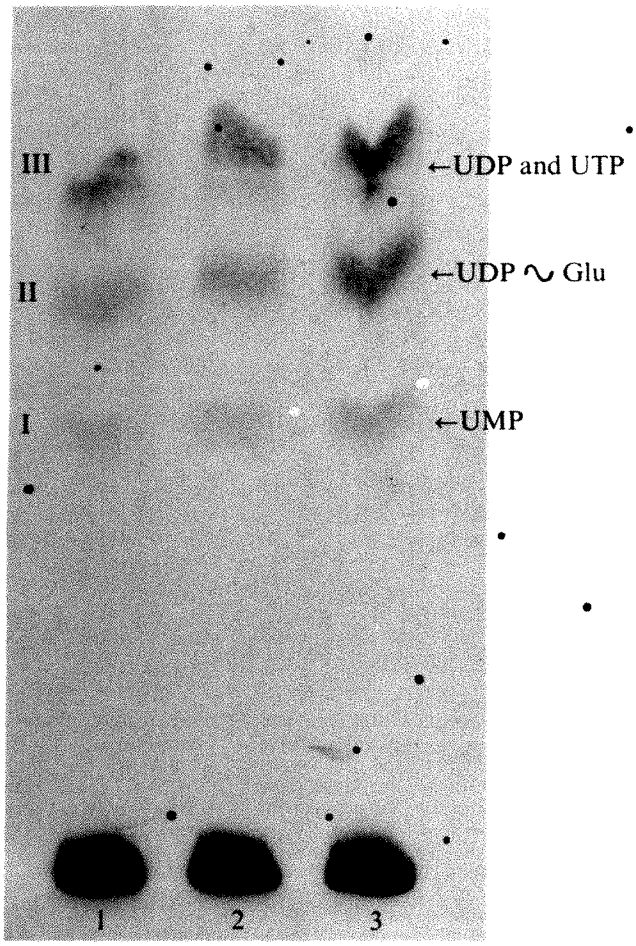

Fig. 5 Analysis of degradation products of reovirus mRNA. Reovirus mRNAs labelled internally with α-^{32}P-UMP were incubated for 10 min in wheat germ extract in the conditions described for Fig. 4*b*. Radioactive materials were recovered from reaction mixtures by phenol extraction and analysed by high voltage paper electrophoresis (50 V cm^{-1}, 50 min) in 10% pyridine-acetate buffer (*p*H 3.5) with marker compounds. Total radioactivity shown in the autoradiogram in tracks 1, 2 and 3 was 110, 120, and 108 × 10^3 c.p.m., respectively. The distribution of radioactivity in each track determined by direct counting of each spot after cutting the paper, was: track 1—origin 87%, band I, 1.7%; band II, 5% and band III, 6.3%. Track 2—origin 83%; band I, 2%; band II, 6.6% and band III, 8.4%. Track 3—origin 60%; band I, 5%; band II, 15% and band III, 20%. Reovirus mRNAs in tracks 1, 2 and 3 contained 5′-terminal m^{7}GpppG^m, GpppG, and ppG, respectively. UDP ~Glu is an abbreviation for uridine diphosphate glucose.

mRNA stability, but translation in wheat germ extract depends on the presence of the methylated blocked structures, m^{7}GpppG^m. The more rapid degradation of unblocked reovirus mRNAs, which have the same sequence and size as blocked or capped mRNA but a different 5′-terminal structure, suggests that mRNA is degraded exonucleolytically from the 5′-end.

5′-Exonuclease cleavage of unblocked mRNA

Reovirus unblocked mRNA was less stable than 5′-blocked mRNA in microinjected oocytes or in wheat germ cell-free extract (Figs 1, 4*b*). When unblocked radioactive mRNA was re-extracted after incubation for 8 h in oocytes, the radioisotope was present in full-size mRNA and in low molecular weight degradation products (Fig. 2*b*). The same results were obtained when mRNA was incubated in wheat germ extract for 10 min and subsequently analysed by velocity sedimentation in a glycerol gradient (Fig. 2*d*). Little radioactivity was observed in products of intermediate size and most of the degraded material sedimented at the top of the gradient. The low molecular weight products were characterised by paper electrophoresis (Fig. 5). Radioactive material analysed after incubation of ^{32}P-labelled mRNA in wheat germ included the mRNA at the origin and three fast-migrating bands but no intermediate spots in the position of oligonucleotides, CMP, AMP, or GMP. The compound in band I corresponds to 5′-UMP. Band III was eluted and identified as a mixture of UDP (32%) and UTP (68%) by paper chromatography with marker compounds in isobutyric acid–0.5 M NH$_4$OH (10:6 v/v). The component in band II migrated in the position of UDP-glucose or UDP-galactose and the ^{32}P-phosphate present in this material was resistant to hydrolysis by alkaline phosphatase. We have not identified the compound(s) in band II, but it has a net charge of −2 by DEAE-cellulose chromatography and can be enzymatically synthesised from ^{3}H-UTP in wheat germ extract, consistent with it being a UDP-sugar derivative. In addition, there is a clear precursor–product relationship between the 5′-UMP (band I), the UDP and UTP (band III) and the presumptive UDP-sugar (band II). When ^{3}H-labelled 5′-UMP (360 pmol, 15 μCi) was incubated for 20 min in wheat germ extract, 54% of the input radioactivity was incorporated into the presumptive UDP-sugar compound and 29% into a mixture of UDP and UTP. By contrast, the presumptive UDP-sugar yielded no 5′-UMP, UDP, or UTP on incubation in wheat germ extract. Thus, the primary degradation product of mRNA labelled with α-^{32}P-UTP was 5′-UMP which in wheat germ was further

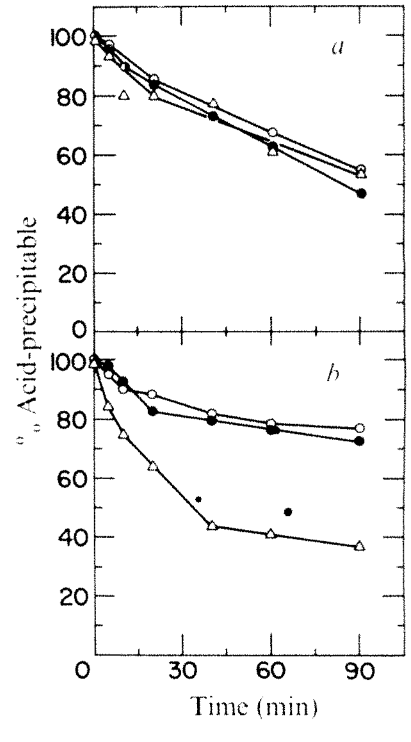

Fig. 6 Test for exonuclease activity in rabbit reticulocyte lysate and mouse L cell extract. Reovirus mRNAs labelled internally with α-^{32}P-UMP (20 μg ml^{-1}, 100–120 × 10^3 c.p.m.) were incubated at 26 °C in conditions used for *in vitro* protein synthesis. *a*, Reticulocyte lysate: the reaction mixture (50 μl) contained 12 mM HEPES buffer (*p*H 7.5), 75 mM KCl, 1.5 mM Mg acetate, 0.8 mM ATP, 0.16 mM GTP, 100 μM Adohcy, 10 mM creatine phosphate, 11 μg creatine phosphate kinase, 5 mM dithiothreitol, 0.1 mM each of 20 amino acids, 20 μg ml^{-1} haemin, 1 μg ^{32}P-labelled reovirus mRNA and lysate equivalent to 2.4 mg protein (provided by W. F. Anderson). *b*, L cell protein synthesising extract: the mixture contained 20 mM HEPES (*p*H 7.6), 120 mM KCl, 5 mM Mg acetate, 6 mM β-mercaptoethanol, 1 mM ATP, 0.2 mM GTP, 100 μM Adohcy, 0.1 mM each of 20 amino acids, 5 mM dithiothreitol, 10 mM creatine phosphate, 10 μg creatine phosphate kinase, and L cell extract equivalent to 252 μg protein (provided by G. Koch). Acid-precipitable radioactivity was assayed as for Fig. 4*b*. mRNAs contained 5′-terminal m^{7}GpppG^m (●), GpppG (○) and ppG (△).

metabolised by the route UMP → UDP → UTP → UDP-sugar. The reovirus mRNA degradation products from *X. laevis* oocytes yielded the same four uridine derivatives when subjected to electrophoresis, and the primary mRNA degradation products in oocytes also are probably 5′-mononucleotides. These observations suggest that the exonuclease processively cleaves unblocked mRNA in a 5′ to 3′ direction yielding 5′-mononucleotides. Confirmation of these characteristics will require studies of the purified enzyme(s).

Like *Xenopus* oocytes and wheat germ extract, L cell protein synthesising extract also contains an enzyme activity that degrades unblocked reovirus mRNA more rapidly than blocked or capped mRNA (Fig. 6*b*). In contrast, we did not detect a differential breakdown of unblocked mRNA in reticulocyte lysate incubated at a 10-fold higher ratio of protein to mRNA than used for L cell extracts (Fig. 6*a*). It will be of interest to determine whether mRNA degrading activities with similar properties exist generally in eukaryotic cells and if so, whether they are involved in mRNA turnover and regulation of cell division and differentiation.

Discussion

In this report, reovirus mRNAs with different types of 5′-termini were used to demonstrate that, in addition to a role in the initiation of protein synthesis, cap structures have an important stabilising effect on mRNA. In accordance with this function, and consistent with the lower stability of unblocked reovirus mRNA in *Xenopus laevis* oocytes and extracts of wheat germ and L cells, a nuclease activity which apparently cleaves unblocked RNA exonucleolytically and probably processively was detected in the amphibian, plant, and mammalian systems. Rabbit reticulocyte lysate was an exception and contained little or no nuclease activity of this type. Assuming that the nuclease activity has a role in mRNA turnover, a highly differentiated cell such as the reticulocyte which has lost its transcriptional machinery might be expected to have no such activity.

In several previous studies, the β-elimination reaction was used to remove m^7G from capped mRNA in order to test its influence on translation *in vitro*. The destabilising effect of unblocking (Figs 1, 2*c*) was then unknown, however. Shih *et al.*[3] compared the efficiency of translation of m^7G-capped and β-eliminated brome mosaic virus RNA and demonstrated that the positive effect of m^7G cap on translation in wheat germ extract was more pronounced at low RNA concentrations ($<20\ \mu g\ ml^{-1}$) than at high levels ($100\ \mu g\ ml^{-1}$) of RNA. The difference in the translational efficiencies might be due to degradation of some of the β-eliminated RNA by wheat germ exonuclease activity. Smith *et al.* and Baglioni *et al.* recently found that the extent of dependence on m^7G for mRNA translation varies with the concentration of potassium ions in protein synthesising reaction mixtures (personal communications). Therefore, in order to understand better the role of the cap structure in the translation of various RNAs, future experiments should be designed to take into account cellular exonuclease(s) and other factors that affect translation in cell extracts.

Polio, encephalomyocarditis (EMC) and satellite tobacco necrosis virus (STNV) RNAs do not contain 5′-cap structures[1]. These RNAs, however, are sufficiently stable to be translated in various model systems and also function as viral messengers in infected cells. How are these uncapped mRNAs stabilised? Allende *et al.*[10] reported that purified ribosomal RNA and 5S RNA are rapidly degraded when microinjected into *Xenopus* oocytes, but globin and *D. discoideum* mRNAs, RNA in ribosomes, and *Escherichia coli* suppressor 3 $tRNA^{tyr}$, which cannot be aminoacylated in oocytes, are all stable. These observations can be explained if extensive and specific secondary structures or the association of proteins, as observed for tRNA, and ribosomes, respectively, prevent 5′-cleavage by exonuclease activity. In this regard, it was found recently that polio virion RNA, the first viral messenger in polio-infected cells, has a viral protein covalently linked at the 5′-end[18]. Poliovirus RNA, EMC virus RNA, polio polysomal RNA, STNV RNA and possibly other messenger RNAs that lack a 5′-5′-inverted structure at the 5′-end may be protected against exonuclease attack by covalent or non-covalent interaction with protein(s) or by 5′-terminal secondary structure. In conclusion, the presence of a blocked or capped 5′-end results in protection of RNA against 5′-exonucleolytic degradation. In some RNAs, for example avian sarcoma virus (ASV) genome RNA which is capped[19] but translated poorly *in vitro*[20], caps may function mainly to confer stability rather than, as with reovirus mRNA, to promote translation. In support of this suggestion, the cap on ASV 35S RNA is not protected against RNase T1 digestion by mouse ascites 40S ribosomal subunits (M. Kozak, unpublished).

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letters to nature

Radio counterpart of the X-ray source 3U0901−09

A RECENTLY published position[1] of the X-ray source 3U0901−09 obtained from the Ariel V experiment overlaps the extent of the Abell cluster A754. Previous radio continuum observations of this area[2] revealed a complicated radio emission distribution. We are mapping a sample of Abell clusters and have made a map of the area containing A754. We find that the position ellipse of the X-ray source is in good agreement with a radio source complex with a very steep spectrum.

The observations were made at 2,695 MHz from the secondary focus of the 100-m radiotelescope of the Max-Planck-Institut für Radioastronomie. The beamwidth at this frequency is

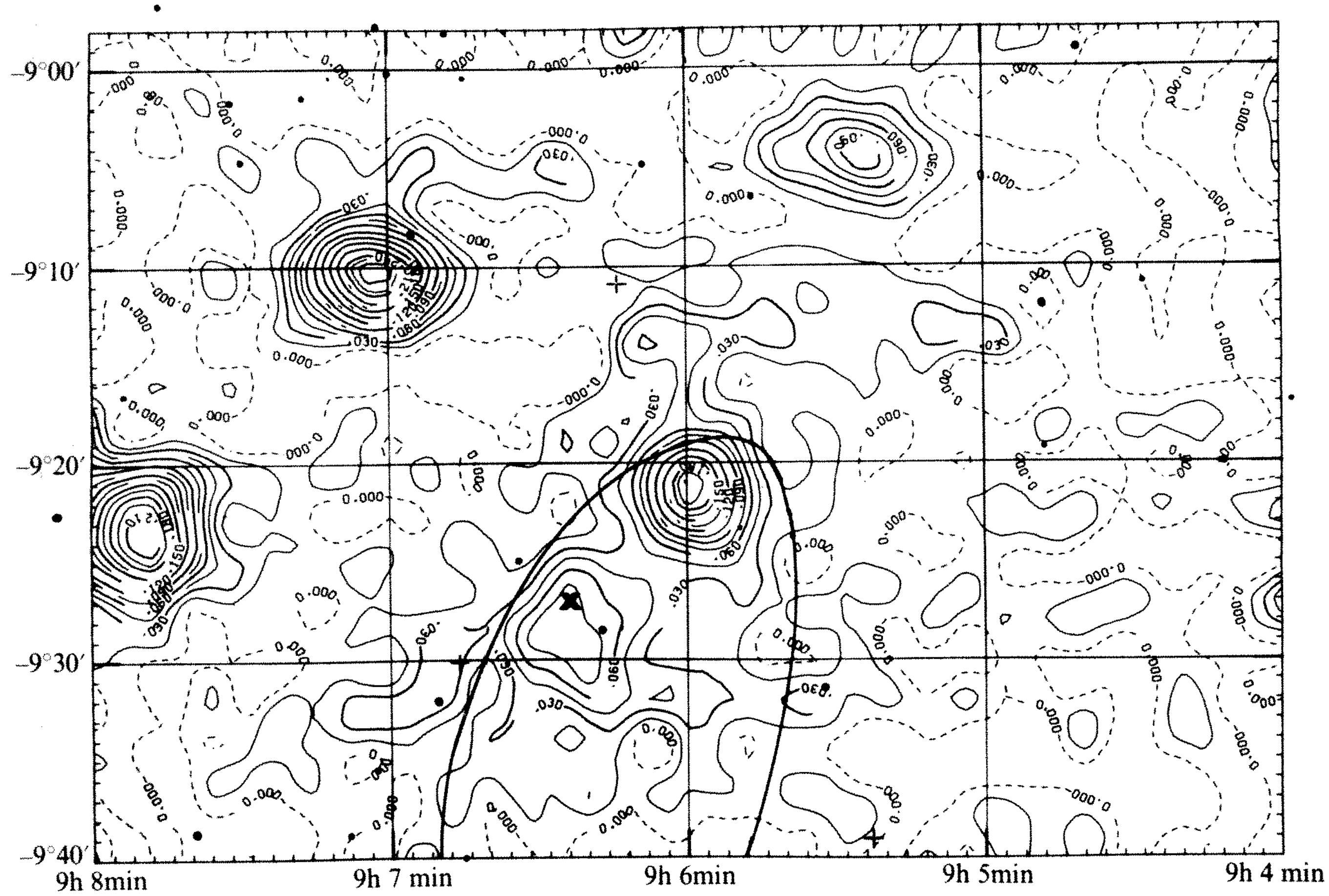

Fig. 1 The 11.1-cm map at an area near A754. Contours are in steps of 15 mJy per beam. Dashed contour shows the zero level. X, centre of A754. The position ellipse of the Ariel V measurement is also shown. Note 1 Jy per beam = 1.5 KT_a = 2.0 KT_{mb}.

4′.4 to 3 dB points. The receiving system is a dual channel correlation radiometer. In this receiving mode a single circular polarisation is combined with the signal from a liquid nitrogen termination in a hybrid, amplified in two phase-stable uncooled parametric amplifiers and correlated at intermediate frequency. The output signal is the difference in power between one circular polarisation and the cold load. The system noise temperature is about 100 K. The bandwidth over which excellent phase stability and tolerable low level of interference are possible is 100 MHz. With this receiver performance fluctuations of 14 mK r.m.s. s^{-1} are expected. In fact, some degradation of performance due to low-level interference and atmospheric fluctuations takes place, requiring an integration of 10 s to achieve 5 mK r.m.s. noise.

Observations were made on 25 April 1976. Scanning of the 42′ × 60′ area was in right ascension at 60′ min^{-1}. Scans were made at intervals of 1′ in declination, requiring 43 steps for the whole map. Two coverages were made and the intensity arrays added to give the map (Fig. 1). Calibration used the source 3C 286 ($S_{11.1}$ = 10.3 Jy) and an internal switched noise diode.

Figure 1 shows four sources, three of which are unresolved by our beam. Extended emission is seen within the position ellipse given for 3U0901 −09 by the Ariel V sky survey instrument[1]. The position ellipse also contains the centre of the Abell cluster A754. Previous observations of A754 were reported by Owen[2] at 1,420 MHz with the 300-ft NRAO radiotelescope. Since his beamwidth was 10′ only one extended source was found at 1,420 MHz in a position which agrees with the centre of gravity of the point source seen at 11 cm in the uppermost end of the position ellipse and the extended source coincident with A754. We have determined the flux of all sources at 11 cm wavelength and have collected our flux values together with Owen's values in Table 1. Our errors in flux determination were estimated to be ±10%. We estimate the error in the 1,420 MHz flux to be ±20% for the point sources and ±30% for the extended one. The spectral index of the two point sources (Nos 3 and 4 in Table 1) is in the range typical for nonthermal radio sources. The spectral index α ($S \propto \nu^{-\alpha}$) of 2.5±0.8 found for the complex which includes the flux of the sources 2a and 2b makes this source similar to other X-ray radio sources or sources in galaxy cluster[3,4,5]. The total flux of the whole complex may be higher than that given in Table 1 due to further emission outside the contours within which we integrate, but it should correspond to the flux which was determined, but it should correspond to the flux which was determined by Owen at 1,420 MHz. In view of this unusually steep spectrum we propose that the X-ray emission comes from this extended radio complex. We have no knowledge of the temporal variations of this source, which may be one of the reasons for this steep spectral index, and we suggest that observations at a number of frequencies should be made over a period of time to finally delineate the spectral index.

Table 1 Flux of sources at 11 am wavelength, and spectral index, α

Source	RA (1950.0)	Dec. (1950.0)	Flux density (mJy) 1420 MHz	2695 MHz	Spectral index α
1	09h05min25.5	−09°04.3′	—	100	
2a	09 05 57.6	−09 21.6	1510	190	2.5±0.8
2b	09 06 24.0	−09 27.0		100	
3	09 07 02.4	−09 10.5	330	285	0.25±0.4
4	09 07 49.1	−09 24.0	440	255	0.8±0.5

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Spectroscopic evidence for interstellar grain clumps in meteoritic inclusions

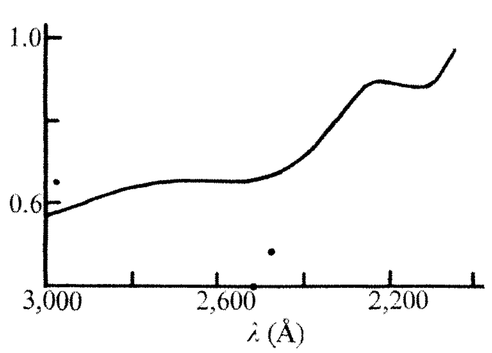
THE occurrence of a primitive, presolar grain component in carbonaceous chondrites has been indicated by several independent lines of evidence, including the discovery of isotopic abundance anomalies[1,2]. The occurrence of organic molecules, including amino acids, in these meteorites is also known[3,4], but their connection with a genuinely pre-solar grain component has been conjectural. We show here that an extract of organic material from the Murchison carbonaceous chondrite has an ultraviolet spectrum with an absorption band centred at $\lambda \approx$ 2,200 Å. The similarity of this feature with the well-known interstellar absorption band at the same wavelength, gives strong credence to an interstellar grain component within this meteorite.

Carbonaceous chondrites contain a significant mass fraction of organic compounds, mainly in the form of aromatic polymers. Typically about 30% of this material is of relatively low molecular weight and could be extracted by using hexafluoroisopropanol as a solvent. The remaining 70% is of higher molecular weight and is usually insoluble[3].

A sample of the Murchison chondrite (supplied by Dr. Onuma) was used to obtain an organic molecule extract with hexafluoroisopropanol. The absorption spectrum of this extract (Fig. 1) shows a conspicuous absorption hump centred at $\lambda \approx$ 2,200 Å, having a half-width $\approx$300 Å and bearing a remarkably strong similarity to the well-known interstellar extinction feature[6–8]. A band of this type is a property of a wide class of organic molecules having conjugated double bonds, for example, $C=C-C=C$, $C\equiv C-C\equiv C$, $C=C-C=O$, with a typical cross-section of $\sim 4 \times 10^{-17}$ cm^2 (refs 9, 10).

Although the 2,200-Å absorption band in the interstellar extinction curve has usually been attributed to graphite particles[11–13], this explanation is unsatisfactory. Spherical or nearly spherical graphite particles of radii $\sim$ 150 Å are required to reproduce the observed features of the astronomical band[14]. Non-spherical particles, or spherical particles with much larger radii will shift the band centre away from λ 2,200 Å, or distort the symmetry of the band. An origin of the interstellar λ 2,200 Å feaure in terms of complex organic molecules lodged in grain clumps would have an advantage in that the mean absorption profile (arising from electronic transitions in an ensemble of molecules) has a central wavelength and width which are independent of the shapes and sizes of the host grain clumps. On the basis of this interpretation, the astronomical and meteoritic spectral data would provide further supporting evidence for a connection between pre-solar interstellar material and inclusions in carbonaceous chondrites.

Fig. 1 The absorption curve of organic material in the Murchison carbonaceous chondrite extracted using hexafluoroisopropanol as a solvent.



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Prebiotic molecules and interstellar grain clumps

INTERSTELLAR molecules detected by radioastronomical techniques in clouds such as OMC 1 and 2 in the Orion Nebula and Sgr B2 in the galactic centre span a wide range of types and complexity. Among the heaviest of the molecules recently discovered is cyanodiacetylene[1] ($H-C\equiv C-C\equiv C-C\equiv N$). There have been earlier detections of precursors to the simplest known amino acid glycine (formic acid and methanimine), and probable detections of polyoxymethylene polymers and copolymers[2–5] in interstellar clouds. We discuss here a possible identification of organic molecules of even greater complexity, and its implications for the start of biological activity.

Large departures from thermodynamic equilibrium in the interstellar medium and the co-existence of solid grains, molecules, radicals, ions and ultraviolet photons provide conditions which are ideal for the assembly of 'exotic' molecular species. If clumping of 100 Å-sized dust grains occurred by a process which we have discussed[6], highly complex organic molecules could be formed and become securely trapped during the 'welding' process of smaller grains. Such grain clumps could be widely dispersed amongst the particulate material in the Galaxy, being responsible for most of extinction and polarisation of starlight.

The spectral identification of highly complex organic species in the interstellar medium might seem hopeless. A property common to a wide class of such molecules, however, is an absorption band centred at $\lambda \simeq 2{,}200$ Å with a half-width ~ 250 Å, which arises mainly due to $\pi \to \pi^*$ or $n \to \pi^*$ electronic transitions in conjugated multiple bonds such as:

$$\begin{array}{ll} C=C-C=C & C=C-C=O \\ C=C-C\equiv C & C=C-C=N \\ C\equiv C-C\equiv C & C=C-C\equiv N. \end{array}$$

The absorption occurs due to transitions between electrons in n and π-orbital states to antibonding π^* states[7,8]. Specific examples of such transitions and relevant spectroscopic data have been cited elsewhere[9]. In general, this absorption band occurs in most highly complex organic species, with a typical value of ~ 200–300 Å for the half-width, and with a molar extinction coefficient $\varepsilon \simeq 10^4$ cm^{-1} (g mol l^{-1})$^{-1}$ (refs 7, 9), which implies an absorption cross-section of $\sim 4 \times 10^{-17}$ cm^2. A typical case is the curve for mesityl oxide (Fig. 1).

It is tempting to identify this feature with the well-known 2,200-Å band of the interstellar extinction curve. The smeared-out mass density ρ of molecules with molecular weight M required to produce the observed extinction coefficient at 2,200 Å of $\sim$ 1.5 mag kpc^{-1} (refs 10, 11) in the interstellar medium is

$$\rho \simeq 2 \times 10^{-27}(M/100)\,\mathrm{g\ cm^{-3}}.$$

For $M \sim 100$ we obtain a value of ρ which is $\sim 10\%$ of the total available CNO density in the interstellar medium. If this material is assumed to be homogeneously mixed with silicate-type grains of radius a, the condition for optical depth less than unity within a single grain at the band centre is

$$a < \sim 3 \times 10^{-5}\ \mathrm{cm}.$$

It may not be fortuitous that this upper limit is about twice the 'typical radius' required for interstellar silicate grains.

Several amino acids of biological importance have been discovered in carbonaceous chondrites[12] and there has also been an identification which is strongly suggestive of the existence of polyoxymethylene polymers or copolymers in the Allende carbonaceous chondrite[13]. Such polymers could have served as glueing agents for smaller grains in prestellar molecular clouds. It is also of interest that an extract of organic materials from the Murchison chondrite using hexafluoroisopropanol as a solvent showed a 2,200 Å absorption feature, typical of conjugated double bonds of the type considered above, and similar to the interstellar extinction hump at this wavelength[11].

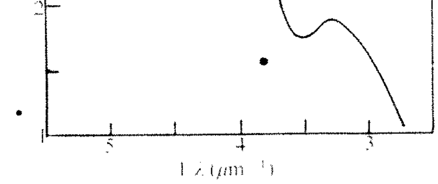
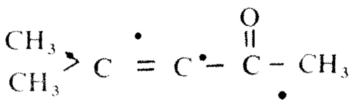


Fig. 1 Molar extinction coefficient ε (cm^{-1} (g mol l^{-1})$^{-1}$) of mesityl oxide



$$\begin{matrix}CH_3 \\ CH_3\end{matrix}\!\!>C=C-\overset{\overset{\displaystyle O}{\|}}{C}-CH_3$$

as a function of inverse wavelength[8].

We tentatively conclude that these data could be interpreted as independent new chemical evidence of the existence of composite grain clumps in the interstellar medium and in carbonaceous chondrites. Moreover, such grain clumps probably include a significant mass fraction of highly complex organic, prebiotic molecules which could have led to the start and dispersal of biological activity on the Earth and elsewhere in the Galaxy.

Even if all the CNO elements on the Earth's surface were initially in the form of the biologically important 20 amino acids, their linkage into feasible peptide chains could not have occurred by chance. Some process of natural selection is required, and this is usually held to have occurred under terrestial conditions. If interstellar grain assembly occurred in prestellar molecular clouds already containing these amino acids, pre-Darwinian selection leading to the production of self-replicable peptide chains, could have depended upon the sticking properties of the various molecules on to the polymeric coatings on 100 Å-sized grains. A welded composite grain with a 'cellular' structure on the scale of $2\pi \times 100$ Å could have served as the host system for the earliest primitive gene in a manner similar to that discussed by Cairns-Smith[15].

We now consider the selective mechanisms which could have operated in the growth and evolution of grain clumps, and their possible bearing on the development of a primitive biological system. Not all polymer coatings have the same sticking probabilities α (ref. 6). Some combinations are more probable than others. Suppose $\bar{\alpha}$ denotes the average sticking probability. What would be the optimum situation for clump growth? A reproducible situation with sticking probability only slightly above $\bar{\alpha}$ would be better than a non-reproducible situation with probability much greater than $\bar{\alpha}$. This is because the latter system, if it occurred, would soon be covered up by further growth of clumps. Once the possibility of a reproducible system is admitted, what reproducible system, among possibly many such systems, would become most widespread? The answer to this question is: a system which also promoted clump division. This leads not only to more clumps but also to a greater total mass of clump material, because of the advantageous surface/volume ratio.

Lastly, we turn to the problem of protection of pre-biotic material against external disruptive agencies, for example, ultraviolet light. Whilst this protection could be provided by an outer shield of solid grains, such a system would be biologically inert. A clump stripped of its outer polymeric coating would not be suitable for sticking, even if it was able to preserve itself. A more refined protection system in the nature of a biological cell wall would be much better, since such an outer surface could permit organic molecules to stick, as well as to diffuse through it.

Thus there seem to be two somewhat different types of selective process which could be operative under interstellar conditions. First, a competition for clump growth in the absence of disruptive agencies, for example, within a pre-stellar cloud; and second, a competition for organic materials (or even for sticking) in a more hostile environment in the presence of disruptive agencies. With the development of a cell wall, the last step could presumably be to 'split out' the inorganic grains which started off the whole process.

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Gravitational radiation detector observations in 1973 and 1974

MARYLAND–ARGONNE gravitational radiation detector observations for 1973–74 have recently been published[1]. This letter contains additional information about how the data were taken and analysed.

A primary goal was to eliminate any unconsciously applied human bias which could affect results. To this end, I removed myself from direct participation in the data reduction beginning in 1972. Mr Michael Lee developed programs for a magnetic tape and computer system of data analysis. A number of checks of the computing were set up. The first check was an independent, analogue experiment, in which data were recorded on pen-and-ink charts, with an on-line computer marking the charts whenever the Maryland and Argonne detectors both crossed threshold within 0.1 s. The pen-and-ink recorder experiment had two outputs, one with, and one without, a time delay introduced into one of the channels before the computer looked for simultaneous threshold crossings. Charts for the data with and without the time delay were marked in coded form (which changed from time to time). Mrs Alessandra Exposito searched the charts for statistically significant coincidences without knowing which charts were which. This analogue procedure found excess coincidences at zero time delay during the same periods that the computerised experiment found them.

In a second checking procedure, Mr William Davis inserted artificial pulses into the gravitational radiation detectors at certain times unknown to the programmer, Mr Michael Lee. Lee correctly identified times when artificial pulses were present and absent. Third, Mr Bruce Webster prepared copies of old data tapes, some identical with the originals and others with one or more channels replaced by the same detector data from another time period. Lee had no knowledge of the information recorded on the magnetic tapes. Lee's analysis correctly identified these tapes.

The telephone circuit from the Argonne detector to Maryland was suspect even though the data were transmitted in coded digital form. The recent paper includes all data for a period when there was no telephone circuit and clock-synchronised recorders were employed at the two detector sites. An excess of coincidences over accidentals was observed during that period.

A major effort has been made to eliminate computing errors. Maryland magnetic tape data were sent to several other research groups together with data on the computer programs and lists of coincidences, in return for raw data from their experiments. During the data exchange, errors were discovered in the Maryland programs and in the procedures of other groups. The Maryland errors were acknowledged quickly and carefully corrected. The differences have now been resolved, and independent groups have confirmed the details of the revised Maryland programming. At the request of two research groups, the data exchange was to be private until agreement could be reached on publication. It is regrettable that incorrect and premature reports on the data exchange have appeared in the literature.

Two of the four authors of ref. 1, Steppel and Lee, independently prepared computer programs to process these data. In summary, results of the magnetic tape computer experiment, the pen-and-ink recorder experiment, and the data exchange indicate that for certain periods there is an excess of coincidences over accidental ones, with significance as great as 5.6 s.d. The use of several independent programs and of the checks in which the searcher did not know what was on the tapes or charts indicates that human observer bias and programming errors have been successfully removed.

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Radiometric measurement of stratospheric HCl

INTEREST in the effects on atmospheric ozone of man-made chlorine compounds has prompted research into their stratospheric chemistry. As HCl is expected to be the major temporary sink for free chlorine in the stratosphere[1–4], measurements of its vertical mixing ratio profile could yield useful information as to the major source of chlorine and its future effects on ozone concentrations. We have used a balloon-borne pressure modulator radiometer (PMR) to measure the atmospheric absorption of solar infrared radiation at sunset, and we report here a vertical mixing ratio profile for gaseous HCl for the altitude range 16–39 km.

The major feature of the PMR is a cell containing gas (HCl, in this case) through which the incoming radiation passes[5]. The gas pressure is modulated at about 19 Hz and the mean pressure in the cell chosen to give maximum modulation of cell transmission at HCl spectral line centres. Thus the incoming radiation is selectively modulated in those narrow spectral regions where atmospheric HCl absorbs, and so the pressure modulator cell acts as a filter of very high resolution ($\sim 4\times10^{-3}$ cm^{-1}).

The front optics of the radiometer is a servocontrolled Sun tracking system built by Denver University[6], which maintains an image of the Sun at the detector. The optical system also contains a rotating chopper which modulates the incoming radiation at 400 Hz and an optical filter which defines a pass band about 300 cm^{-1} wide around 2850 cm^{-1}. This region of the spectrum contains most of the fundamental vibration–rotation band of HCl. The detector, made of lead selenide at ambient temperature, receives radiation which has components at 400 Hz (the wide-band signal), at 19 Hz (the PMR signal) and at 400 ± 19 Hz (the side-band signal). The PMR and side-band signals contain the same information but the latter is used for instrumental convenience. The side-band:wide-band ratio is related to the amount of atmospheric HCl in the path between the instrument and the Sun.

The results presented here were obtained on a flight from Holloman USAF base, New Mexico (32° 52′N, 106° 07′W) on 17 March 1976. The radiometer remained at its float altitude of about 35.5 km until after sunset and was able to take measurements for solar zenith angles ≥90°. In this way the Sun is viewed through a long atmospheric path, enabling low concentrations of trace constituents to be detected. This technique (limb scanning) also allows vertical resolution in mixing ratio profile to be obtained. Signals for zenith angles <90° are used to calculate the level of the level of the side-band:wide-band ratio when there is no atmospheric absorption.

The mixing ratio profile has been derived from the data using a computer model which calculates the signals expected from a PMR for a given atmosphere by performing a line by line numerical integration across a Voigt profile for all the HCl spectral lines in the pass band of the filter. The spectral line strengths and Lorentz halfwidths used are those given by Toth *et al.*[7,8]. The results are shown in Fig. 1. Relative errors are indicated; also, at the present stage of analysis, there may be an absolute error of up to ±20% in the

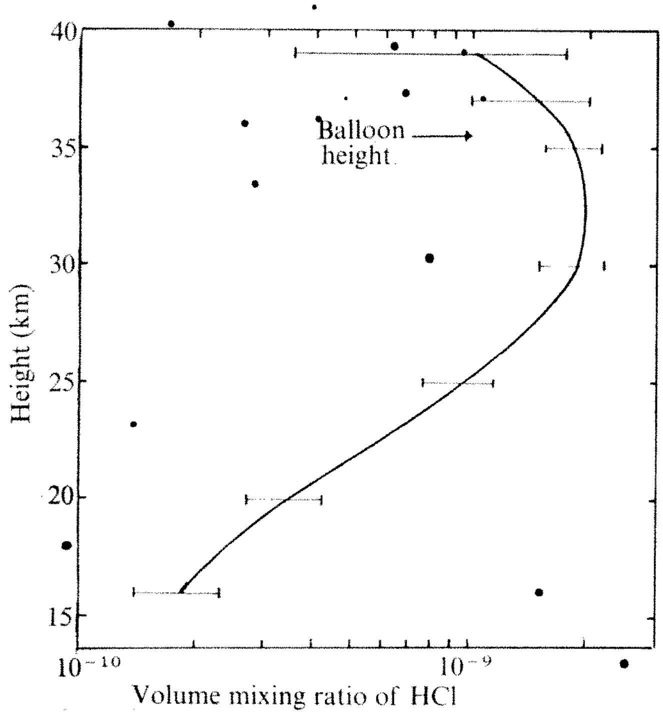

Fig. 1 Vertical profile of the mixing ratio of HCl in the stratosphere calculated from measurements of atmospheric transmission.

mixing ratio. The retrieval takes account of atmospheric temperature structure (measured by radiosondes coincident with the flight) and of the effects of atmospheric refraction. Small corrections have also been made for the estimated effects on the wide-band and side-band signals of other atmospheric constituents which absorb in this spectral region, using the spectral line data tabulated by McClatchey *et al.*[9].

The shape of the mixing ratio profile implies that the major source of HCl in the stratosphere is around 32 km. Other measurements of HCl (refs 10–13) have been based on the measured absorption of individual HCl spectral lines, whereas the PMR uses all the lines in the band. Figure 2 shows that the mixing ratio profile given here is similar to those of Ackerman *et al.*[11] and Williams *et al.*[12] but that its shape is different from the most recent profile measured by Raper *et al.*[13].

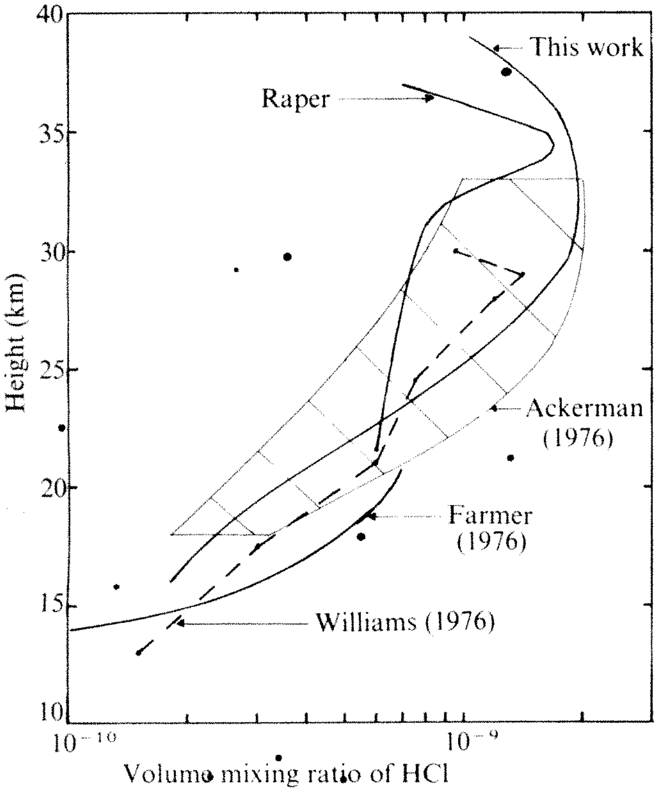

Fig. 2 Mixing ratio profiles of stratospheric HCl. A comparison of the results of this work with other measurements.

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13 Raper, O. F. *et al.* to be published.

Mantle helium in the Red Sea brines

HELIUM isotope studies on terrestrial samples have revealed the existence of two helium components which are clearly distinct from atmospheric helium. The first of these, which we term 'crustal helium', was identified in 1946 in natural gas wells[1]. This crustal component is produced by radioactive decay of U and Th to ^{4}He, with ^{3}He production by (n, α) reactions on Li; the resulting helium is characterised by $^3\text{He}/^4\text{He} \simeq 10^{-7}$, one-tenth of the atmospheric ratio[2]. The second component, 'mantle helium', was discovered as 'excess ^{3}He' in deep ocean water, attributed to a flux of primordial helium from the mantle[3]. Studies of the $^3\text{He}/^4\text{He}$ ratio in deep water on the East Pacific Rise[4] and in helium trapped in submarine basalt glasses[5,6] have shown that this mantle component is characterised by $^3\text{He}/^4\text{He} \simeq 10^{-5}$, about 10 times the atmospheric ratio and 100 times the ratio in crustal helium. Basalt glasses from the Western Pacific Lau Basin, the East Pacific Rise, and the Mid-Atlantic Ridge contain trapped helium with similar $^3\text{He}/^4\text{He}$ ratios, indicating that mantle helium in at least three areas in which new lithosphere is being formed has a unique and uniform isotopic signature.

The hot brines in the axial rift of the Red Sea[7] provide an opportunity to sample helium at another plate margin, and to determine the extent to which these brines have interacted with mantle-derived material. Isotopic and chemical studies of the brine water[8,9] have shown that the source water for these geothermal brines is ocean water of normal salinity which circulates downward through evaporites and horizontally along fissures in the axial basalts to reach the brine-filled depressions. We now report measurements of helium and neon which reveal additional information on the origin of these brines and their relationship to the Red Sea rift.

Our samples were collected on the 1966 Woods Hole CHAIN-61 expedition, using a Nansen-bottle 'piggyback sampler' which recovers samples isolated at *in situ* pressure in copper tubing[10]. We analysed two samples taken 2 m apart from the Atlantis II Deep (Station 714, 21°20.5′N, 38°04.5′E); the bottom bottle was 43 m above the bottom in the middle of a 56 °C, 126-m thick layer. From Discovery Deep we analysed three samples collected over a 7-m interval (Station 717, 21°16.7′N, 38°02′E); in this case, the bottom bottle was 42 m

Table 1 Helium, neon and ^{3}He/^{4}He in Red Sea brines (CHAIN 61 Expedition)

	Depth (m)	Concentrations (10^{-6} cm^3 STP per g H_2O) Helium	Neon	He/Ne	(^{3}He/^{4}He) × 10^6	Concentration per atm solubility ^{3}He	^{4}He	Ne
Atlantis II								
714–G7	2121	14.0	0.14	100	12.1	3260	370	1.0
Discovery	2123	(14.8)	—	—	12.2	3470	390	—
714–G8								
717–G10	2153	(14.2)	(0.15)	95	—	—	380	1.0
717–G11	2158	14.1	0.14	105	12.0	3250	370	0.9
717–G12	2160	(13.4)	—	—	12.2	3140	360	—
Red Sea surface water		0.038	0.15	0.25	1.38	≡1	≡1	≡1

Concentrations in brine samples are accurate to 7% except for values shown in parentheses which are accurate to ~ 15%. Values for surface seawater (28 °C, 38‰ salinity) are for solubility equilibrium with air at 1 atm (ref. 11). Helium isotope ratios are accurate to 5% and are reported relative to the ratio 1.40 × 10^{-6} for atmospheric helium[12]. Sample designations refer to cast and bottle number.

above the bottom in the 44 °C, 164-m thick layer. Conductivity measurements aboard ship showed that the salinity of each sample was the characteristic brine value of 257.5 g kg^{-1} (ref. 9). The samples, each about 20 cm^3, were sealed in the copper tubing with refrigeration clamps and stored at 4 °C until analysed. They were collected at the same locations as those analysed previously for N_2, Ar and total CO_2 (ref. 10).

Gases were extracted in a vacuum line made of Corning 1720 glass and stainless steel. In vacuum all samples were clear and colourless, indicating that no contact with oxygen had occurred[10]. The extraction and rare-gas purification procedure, which has been tested extensively on seawater samples, collects > 99.9% of the He and Ne. The total system and spectrometer blank (in cm^3 STP) is 9 × 10^{-15} for ^{3}He, 1.3 × 10^{-9} for ^{4}He, and 1 × 10^{-10} for Ne, < 0.1% of the sample in each case. ^{3}He, ^{4}He, and Ne were measured on a double-collecting mass spectrometer[5] which completely resolves ^{3}He from HD + H_3. A charcoal U-trap chilled at −195 °C was included in the static volume during analysis to reduce interference from Ar^{2+} at mass 20. The ^{3}He/^{4}He ratio was measured in double-collection mode, and the neon was then measured by voltage switching. Air standards were used to calibrate the helium isotope ratios and the absolute amounts of He and Ne as determined by peak height measurements.

The results, summarised in Table 1, show that both brines are highly enriched in helium with a mantle-helium isotopic signature. Because the large variations in salinity in the 15 known brine pools[13] are due to addition of salt from evaporites and dilution by mixing with seawater[9], concentrations of gases and their enrichments relative to solubility equilibrium with the atmosphere are best expressed in terms of gas to water ratios. The measured He concentrations (~ 10.5 × 10^{-6} cm^3 STP per g of brine) are thus given as He/H_2O ratios in Table 1 based on a salinity of 257.5 g kg^{-1}, and are compared with atmospheric-equilibrium solubility ratios in Red Sea surface water at 28 °C, 38‰ salinity, the source-water values estimated from stable isotope ratios[8] and dissolved Ar in Atlantis II brine[10]. On this basis the Atlantis II and Discovery brines are 380 times supersaturated in helium. The ^{3}He supersaturations are even more spectacular: a factor of 3,300, which can be compared to the highest ocean water enrichment factors of 1.5 observed in deep water adjacent to the East Pacific Rise[1]. In contrast to these large helium anomalies, the neon concentrations for both brines agree within errors with equilibrium values, indicating neon enrichments of < 10%.

The ^{3}He/^{4}He ratio in these brines, 1.2 × 10^{-5}, is similar to that in helium in the glassy rims of recent tholeiitic basalts on Atlantic and Pacific rises[5,6] (Fig. 1). Since helium in evaporites should be pure 'crustal' helium with a ratio ≃ 10^{-7}, we conclude that the helium in these brines is primordial helium extracted from basalts in the axial rift during subsurface flow of brine from evaporites to the brine depressions. Comparison with the enrichment factors in brines against original seawater for other elements[9] shows that ^{3}He ranks with the trace metals Pb, Mn, Fe and Zn, as the only elements exhibiting enrichments of a factor of > 1,000 in these brines; ^{4}He is next in order (enrichment ≃ 400), and all other species are enriched by factors < 100. Both the trace metal and major element enrichments are entirely consistent with derivation from evaporites, as shown by comparison of added salts in Red Sea and known

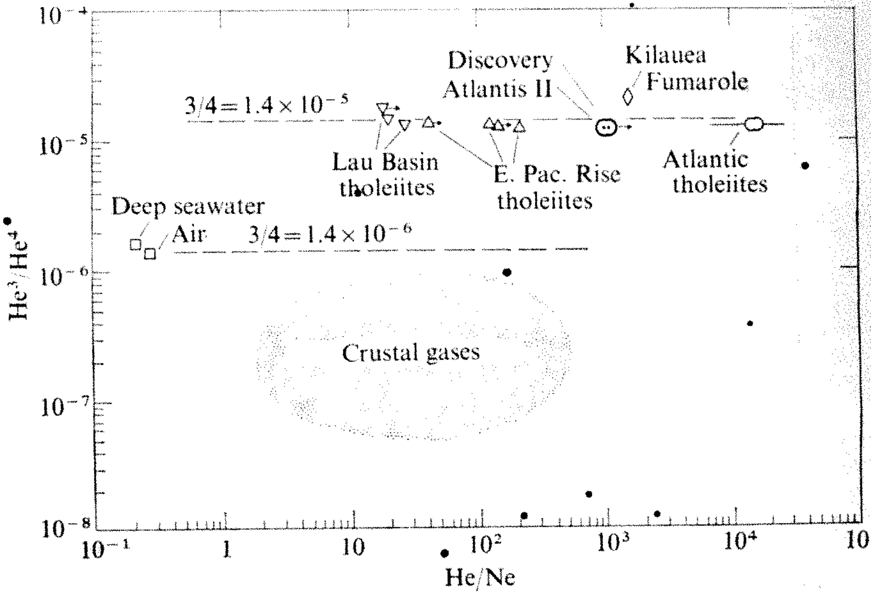

Fig. 1 ^{3}He/^{4}He against He/Ne ratios in Red Sea brines, tholeiitic basalts, Kilauea volcanic gas, and crustal gases. Arrows on points signify lower limits for He/Ne ratios.

salt-deposit brines[9]. In contrast, the similarity of helium isotope ratios in fresh basalt glasses and in the brines indicates that $> 80\%$ of the brine helium must have been extracted from basalts. Helium is thus the only component in these brines which has so far been shown to require a derivation from a source other than the Red Sea evaporites.

Comparison with helium concentrations in recent basalt glasses[5,6] indicates that the He/H_2O ratio in these brines is equivalent to a basalt/H_2O ratio of the order of 2–10 g per g for complete extraction. The slightly lower ^{3}He/^{4}He ratios in the brines than in the basalt glasses may be attributed to a 10–20% dilution with radiogenic helium produced in the evaporite deposits by decay of U and Th. Weiss[10] showed that the Ar/H_2O ratio in these brines is close to the original solubility ratio. As the ^{4}He/^{40}Ar ratio in fresh basalts is about 8 (refs 14, 15), $< 1\%$ of the argon in these brines has been derived from basalts.

Figure 1 shows the ^{3}He/^{4}He and He/Ne ratios for the two brine samples compared with the values for air, crustal gases[16], tholeiite basalt glasses and a gas sample from Kilauea volcano[6]. For the brines we have estimated the He/Ne ratio in the 'excess' component above atmospheric solubility by assuming that the neon excess is $< 1.4 \times 10^{-8}$ cm^3 STP per g H_2O for both brines, that is, 10% of the measured neon concentration. For the injected component in the Atlantis II and Discovery brines, this estimate yields a lower limit for the He/Ne ratio of ~ 1,000; this value, plotted in Fig. 1 shows that Red Sea basalts are more similar in He/Ne ratio to Atlantic than to the Pacific basalts so far analysed.

The relationships in Fig. 1 show that the ^{3}He/^{4}He ratio is essentially identical in samples from four plate margins and three oceans in which the He/Ne ratios vary by a factor of 1,000. These large He/Ne variations are due either to an intrinsic heterogeneity in mantle primordial gas concentrations or to a He–Ne separation process which does not fractionate helium isotopes significantly[6]. Helium from the Red Sea brines and other plate margin basalts is also distinct from Kilauea helium, which has a 3/4 ratio ~ 15 times atmospheric, an indication that Kilauea may be tapping a different part of the mantle, more enriched in ^{3}He.

That ^{3}He and ^{4}He are the only Red Sea brine components which can be shown unequivocally to be derived from hydrothermal circulation in basalts, rather than in evaporites, has interesting consequences for exploration of possible hydrothermal systems in ocean-ridge basalts[17]. The ~ 3,000-fold enrichment of ^{3}He in Red Sea brines relative to seawater shows that ^{3}He (and total helium) should be by far the most sensitive indicators of hydrothermal emanations from ocean-floor basalts. We are now using helium isotope measurements in conjunction with a deeply-towed sample collection system to map hydrothermal plumes in active spreading centres[18].

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The continental crust northeast of Newfoundland and its ancestral relationship to the Charlie Fracture Zone

CONTINENTAL edges that are matched in pre-continental drift reconstructions have generally been defined by an isobath. When the isobath chosen is too shallow, isolated bathymetric highs within the 'oceanic' area are interpreted as continental fragments that may consequently be moved at will to fill any gaps in the reconstruction[1]. Orphan Knoll (which had a history of uplift, erosion and subsidence[2] and Flemish Cap[3] are two such fragments for which lateral movements across the northeast Newfoundland shelf have been invoked[4]. Detailed bathymetric, magnetic and gravity surveys[5,6] have confirmed that the entire intermediate depth area of the northeast Newfoundland Shelf inshore of Orphan Knoll and Flemish Cap is continental in nature thereby invalidating reconstructions which assume otherwise. I show here that the offshore extension of the Hermitage–Dover fault in Newfoundland provided the line of weakness along which the Charlie transform fault developed.

Arcuate zones of magnetic anomalies dominate eastern Newfoundland and the northern Grand Banks[7] (Fig. 1). In the south central Grand Banks, the magnetic highs correlate with basement highs (Fig. 2) from which pre-Devonian metasedimentary rocks[8] were recovered. Similar rocks on Avalon Peninsula overlie the Precambrian volcanics responsible for the onshore portion of the magnetic highs. These arcuate 'continental' magnetic zones extend into the intermediate depth (1000 m) area of the northeast Newfoundland Shelf (Figs 1 and 2), strongly suggesting that it is underlain by continental crust. In support of this, continental basement can be traced westward along the reflection profile indicated in Fig. 2, from continental Orphan Knoll to the eastern edge of a deep sedimentary basin[9]. A similar seismic reflector can be traced eastwards from land in an entirely continental sequence to the western edge of the same basin[9]. The only possible discontinuity in continental basement from seismic evidence, therefore, underlies the sedimentary basin. But, one of the bands of high magnetic field originating onshore in Precambrian volcanic rocks crosses the basin at the same location as the seismic reflection profile (Figs 1 and 2), thereby bridging the gap. Continuity of continental crust from Newfoundland out to Orphan Knoll is, therefore, corroborated.

The continental area of the northeast Newfoundland Shelf seaward of approximately the 300-m isobath has, however, subsided with respect to the Grand Banks[7]. Although its southern fractured margin is clearly marked by an east–west line of magnetic anomalies at 48 °N (Fig. 1), the overall line of dislocation is marked by a gravity high (Fig. 3). This is partially due to the localised sedimentary load on the lithosphere[11] but the magnitude of the gravity anomaly can only be explained if the subsided crust has increased in density or has been thinned as a result of margin tectonics (R. A. Folinsbee, personal communication). The inverse correlation between changes in the gravity and magnetic field, which is evident on a local scale over the basin that lies along the line of dislocation, results from the thicker sedimentary section giving an increased load and higher gravity while increasing the depth to basement and, therefore, reducing the magnetic field.

Although the magnetic pattern of Flemish Cap contrasts with that of the Grand Banks, continental basement can be traced between them except for a 40-km wide zone in Flemish Pass[3]. This distance is, therefore, the maximum possible extent of crustal fracturing or thinning east of the Grand Banks. An overwhelming majority of the area between Orphan Knoll, Flemish Cap and Newfoundland is, therefore, continental, and no major lateral

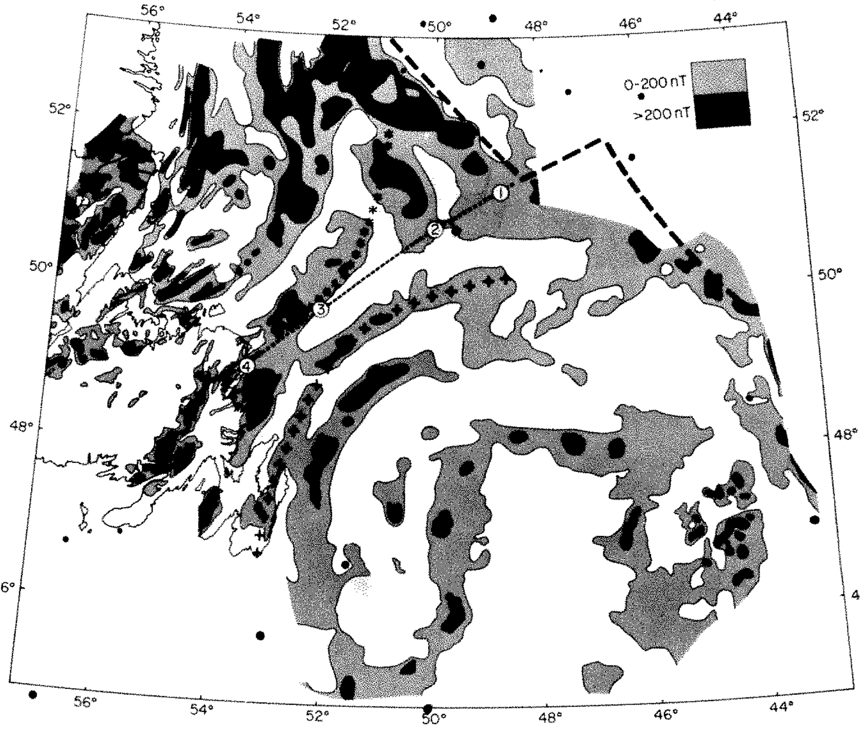

Fig. 1 The magnetic trends of the continental shelf northeast of Newfoundland based on tracks with maximum spacing of 10 km (refs 5,6). - - - -, Line marking 1 offset of edge anomalies, 2 termination of N.W.-trending anomaly pair, 3 divergence between offshore extension of Avalon and Gander (magnetic) trends, and 4 Dover Fault. ****, Localised magnetic trend obliquely crossing N.W.-trending anomaly pair. + + + +, Magnetic trend extending Precambrian volcanics beneath sedimentary basins crossed by Grant[9] seismic reflection line (see fig. 2). ––––, Ocean–continent boundary.

adjustment of the positions of Flemish Cap or Orphan Knoll has occurred. This requires that the continental edge used in palaeogeographic reconstruction should lie east of Orphan Knoll and Flemish Cap as indicated in Figs 1, 2 and 3.

An offset in oceanic magnetic lineations either side of the Charlie Fracture zone[10] correlates well with the northern edge of the subsided northeast Newfoundland shelf and with an offset of approximately 100-km in the gravity and magnetic 'edge anomalies' at the continental margin (point 1 in Fig. 1)[6-12]. This suggests that the Charlie transform fault can be related directly to an initial offset in the Canadian margin[10]. Attempts to identify the seaward extension of one of the faults in Notre-Dame Bay as the ancient line of weakness[13] controlling that transform fault[14,15] are only substantiated as far east as 52 °W (refs 9, 16), whereas the older and more fundamental crustal boundary represented in Newfoundland by the Hermitage–Dover Fault can be traced across the shelf to the inshore end of the Charlie Fracture Zone, by a series of disruptions in the geophysical data (points 1–4, Fig. 1).

A pair of positive and negative magnetic anomalies with a northwesterly trend, 100-km long and centred at approximately 51 1/2 °N, 50 1/2 °W, must overlie continental crust because they are superimposed by subsidiary N–NE trending anomalies that can be traced to pre-Mesozoic features ashore (Fig. 1). 'Basement' velocities of 5.1–5.3 km s^{-1} obtained over this region have been interpreted as indicating a decrease in the effect of the Acadian orogeny northeast of Newfoundland[17]. On the basis of correlation

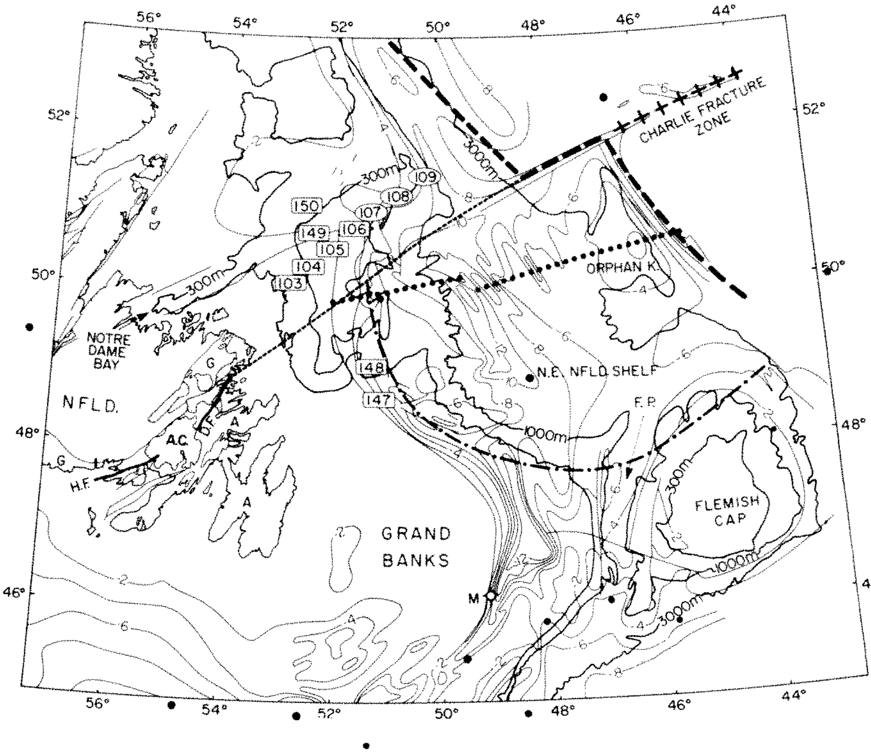

Fig. 2 Contours on basement (km) (refs 9,10) and bathymetry of the Newfoundland continental margin. – – – –, Ocean–continent boundary. –·–·–, Inshore edge of subsided crust beneath NE Newfoundland Shelf . . . ., Grant[9] seismic reflection line. A.C., Ackley City granite batholith; D.F., Dover Fault; H.F., Hermitage Fault; A., Avalon zone; G., Gander Zone; F.P., Flemish Pass. Numbered rectangles indicate location of refraction lines[17] with high basement velocity; numbered ellipses indicate low basement velocity.

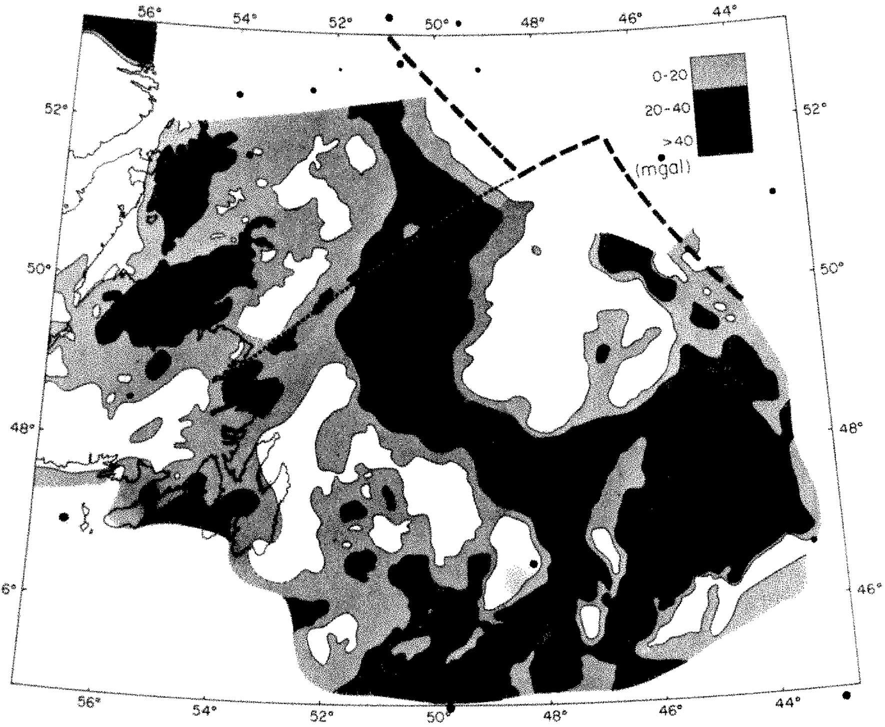

Fig. 3 The gravity trends of the continental shelf northeast of Newfoundland. Bouguer anomalies on land and free air anomalies at sea. Ocean–continent boundary and Dover–Charlie Fault Zone line from Fig. 1.

with magnetic field trends, however, the adjacent higher (Acadian?) velocities (5.7–6.3 km s^{-1}) probably extend to the continental margin north of the refraction lines. The lower velocity area has in fact subsided along faults subparallel to the continental margin as indicated by the change in depth to seismic basement (Fig. 2) and the increase in wavelength of the transverse magnetic anomalies. The northwest-trending anomalies, which reflect this basement faulting, are abruptly terminated along the southwestward projection of the trend of the Charlie Fracture Zone (2 in Fig. 1).

Further projection of this trend southwestward follows the boundary between the contrasting magnetic anomaly patterns of the Avalon and Gander tectono-stratigraphic zones[18]. The arcuate magnetic trends of the Avalon Zone and the Grand Banks veer steadily to the east while the trends of magnetic anomalies emanating from the Central Mobile Belt veer gradually northward (3 in Fig. 1).

The calculated depths to an intermediate crustal layer of velocity 6.9–7.6 km s^{-1} (ref. 17) can accomodate a 2-km normal fault[14] between refraction lines 148 and 149 (Fig. 2). The reduction in amplitude and increase in wavelength of magnetic anomalies that would be associated with such a fault, are found at the eastern edge of the high magnetic zone that enters Newfoundland on the western side of the Dover Fault (3 and 4 in Fig. 1).

The Dover[19] and Hermitage Faults[20] define the boundary between the Gander and Avalon Zones (Fig. 2). The fundamental nature of this boundary established on land, combined with the ability to trace its extension, by means of geophysical boundaries (1–4, Fig. 1), to an offset in the continental margin and the western end of the Charlie Fracture Zone, indicates that the Hermitage–Dover Fault Zone is ancestral to the Charlie Fracture Zone.

Cherkis[15,21] has suggested from magnetic field data that the line of weakness along which the Charlie transform fault developed is also the location of the Hercynian (Variscan) Front. Although magnetic trends in Europe have been interpreted as associated with Hercynian deformation[22], the seaward extension of any trend (magnetic or gravity) that might correlate with the Hercynian Front itself, is not apparent west of Ireland[23,24]. On most palaeogeographic reconstructions of the North Atlantic, however, those magnetic trends in Europe attributed to Hercynian deformation are roughly concentric with the arcuate magnetic zones of the Grand Banks and the Avalon Zone (Fig. 4). If the Hercynian Front lies at the northern limit of those magnetic zones, as in Europe, the implication would be that the Hercynian Front follows the Hermitage–Dover Fault Zone, but this suggestion can be opposed on geological grounds. Work in progress on clearer identification of those potential field trends associated with strictly Hercynian features in North America and Europe indicates that the arcuate zones in Newfoundland and Iberia are Precambrian rather than Palaeozoic. Hercynian trends would, therefore, be associated with disruption of the magnetic pattern rather than the pattern itself.

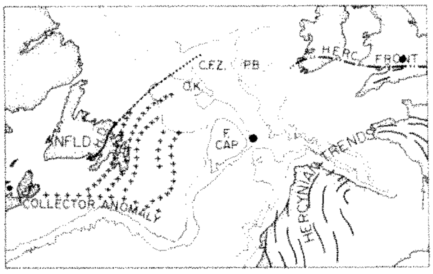

Fig. 4 Trans-Atlantic correlation between north-east Newfoundland and Hercynian trends. The Collector Anomaly[7] marks the southern limit of the Avalon arcuate magnetic zones. Juxtaposition of N. America and Europe is based on a morphological fit between the two margins in which no movement of continental fragments has been closed[25]. No other justification is claimed for this reconstruction, and the roughly concentric nature of trends prevails if other reconstructions are used. C.F.Z., Charlie Fracture Zone; O.K., Orphan Knoll; F. Cap, Flemish Cap; P.B., Porcupine Bank.

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Preliminary palaeomagnetic results from Makapansgat and Swartkrans

THE South African hominid sites have proved difficult to date since radiometric and fission track methods have yielded no results, and faunal and geomorphological methods provide only wide limits. We describe here preliminary palaeomagnetic results which suggest that polarity stratigraphy can be used at these sites, and which indicate an age of between 2.8 and 3.3 Myr for Member 2 of the Makapansgat Formation.

Samples were collected at the Swartkrans and Makapansgat Limeworks sites. Both sites were described by Brain[1] who has subsequently revised his interpretation of Swartkrans[2] and in conjuction with Butzer has suggested new nomenclature[2,3]. Detailed mapping at Makapansgat by one of us (T.C.P.) has led to a revision of the stratigraphy which will be briefly described here. Full details will be published elsewhere.

The Makapansgat Formation constitutes the cave filling at the Makapansgat Limeworks hominid site, the cave being a solution cavity in the Malmani Dolomite of the Transvaal Supergroup. The lowest member (Member 1) is a contaminated travertine, locally bone-bearing, deposited on an irregular dolomite floor and locally tilted and distorted. Member 2, which forms the lower part of Brain's Lower Phase 1 unit[1], is a bone-bearing brownish-red well-bedded deposit of silt to fine sand grade material coarsening upwards, and is 2–10 m thick. It was deposited subaqueously within the cave and there seem to have been two depositories, one to the east and one to the west, separated by a floor high but controlled by a common water table level close to 1458-m elevation. Member 3, formerly the Grey Breccia of Lower Phase 1, is a thin (0.5–2.0 m) deposit consisting largely of a densely packed accumulation of bone cemented by calcite. Much of the Makapansgat fauna is derived from this Member which unconformably overlies Member 2. Member 4, formerly the Pink Breccia of Upper Phase 1, is a thick (3–15 m) deposit of pinkish silty sand material containing many angular chert and dolomite fragments, the larger of which are probably derived from roof falls, and local concentrations of bone. Member 5 (formerly the Red, or Phase II, Breccia) constitutes a locally well-stratified brownish-red silty sand matrix containing abundant chert and dolomite fragments and resting unconformably upon an eroded surface of Member 4. This unconformity probably represents a considerable lapse of time. Bone, often poorly preserved, is scattered throughout Member 5. Most of the remains of *Australopithecus africanus* were recovered from Member 3, but the posterior part of one cranium was recovered from Member 4, and one equivocal maxillary fragment from Member 5.

Forty palaeomagnetic samples were collected. The very varied physical nature of the rocks dictated a variety of collecting techniques. At Swartkrans 15 block samples, oriented magnetically, were collected. At Makapansgat 18 samples were cored and four (all from Member 1) were collected as blocks; all of these were oriented with a theodolite using Sun sights. Three samples from soft red muds (Member 2) in the deepest part of the cave were encased in small plastic cubes and oriented magnetically. Member 3 was not sampled, due to the poor mechanical properties of the rock at the accessible localities. All measurements were made in the Salisbury laboratory using a spinner magnetometer described by van Oorschot and Ridler[4]. Routine AF cleaning was used and a stable component was isolated at typical peak fields of about 20 mT; many samples showed evidence of rotational remanent magnetisation[5] at higher fields. Stable directions were arbitrarily classified as normal (N) or reversed (R) if they were within 40° of the relevant dipole field direction and as intermediate (I) if they fell outside this limit. Samples which showed erratic demagnetisation, or which exhibited internal disagreement between specimens were classified as giving no result (X).

The results for Swartkrans are shown in Table 1. One sample broke up during preparation and only 14 are shown in the table. Of these, eight are unstable or inconsistent, four are intermediate and two are normal. The large number of unstable samples is disappointing and the proportion of intermediate samples is higher than would be expected for a geomagnetic field effect. Even if the two normal samples represent an original primary magnetisation we do not feel that any chronological conclusions should be drawn from these data.

Table 1 Polarity data for Swartkrans

Unit	Sample No. (PM)	Polarity of samples
Member 1, Unit B1 Pink Breccia	1, 2	X, I
Member 1, Unit B2 Pink Breccia	3, 4	I, I
Member 1, Unit C Orange Breccia	5, 6, 7	X, X, X
Member 2, Inner Cave Stratified Brown Breccia	8, 9, 10, 11	I, X, X, X
Member 2, Outer Cave	13, 14, 15	N, X, N

Samples from Makapansgat showed much greater stability: the results are given in Table 2 and shown schematically in Fig. 1. Member 1 is entirely reversed. The beds at the point of sampling are tilted and when the palaeomagnetic directions are corrected for the tilting they become significantly more scattered (although still reversed), suggesting that the magnetisation was acquired after tilting. Member 2 is horizontally bedded and lies with minimum distortion upon the tilted Member 1, indicating that the deposition of Member 2 also occurred after the tilting. In Member 2 the lowest sample in the western depository is intermediate, whilst the remaining 5 horizons (containing 12 samples) are all normal. Low in Member 4 an intermediate horizon occurs, while high in Member 5 two reversed horizons occur followed closely upwards by two intermediate horizons.

The simplest interpretation of these results is that there is an upwards reversed to normal transition low in Member 2 which is otherwise entirely normal. An intermediate horizon low in Member 4 suggests a transition back to normal, but since Member 3 was not sampled it is not clear whether this is the transition immediately following that in

Table 2 Polarity data for Makapansgat

Unit	Sample No. (MT) by horizons	Polarity of horizon
Member 5	25	I
	24	I
	23	R
	18, 19	R
Member 4	20, 21, 22	I
Member 2, east	15, 16, 17	N
	12, 13, 14	N
Member 2, west	8, 9	N
	5, 6, 7	N
	10	N
	11	I
Member 1	2	R
	4	R
	3	R
	1	R

Member 2. A reversed section high in Member 5 followed by two intermediate samples suggests another reversed to normal transition. The placement of these polarity changes in the polarity time scale must depend upon other evidence for the approximate age of the beds.

Two other lines of evidence are available. The first is geomorphological. Partridge, using estimates of nickpoint migration and valley widening, calculates an approximate age for the cave opening at Makapansgat of 3.7 Myr (ref. 6). This is the time from which cave filling became possible, and is therefore a maximum age for the deposits. The second is faunal. Cooke[7] has used suid evidence to suggest that the Makapansgat fauna is "in the vicinity of 2.5 to 3.0 million years old" (quoted by Tobias[8] in a review of the evidence). White and Harris[9] have recently suggested that "the Makapansgat (suid) fauna is equivalent in age to Members B–C of the Shungura sequence" in Ethiopia. The palaeomagnetic stratigraphy of Shuey *et al.*[10] for the Shungura Formation places the base of Member B, the base of Member C and the top of Member C at about 2.9, 2.6 and 2.4 Myr, respectively. Vrba has used bovids to suggest tentatively that the Sterkfontein type site is "somewhere between 2 and 3 Myr" in age (personal communication) whilst Wells[11] feels that the Makapansgat fauna slightly predates that of Sterkfontein. The published faunal evidence is still rather scanty, but seems consistent with an age of between 2.5 and 3.0 Myr for Member 3, from which the bulk of the fauna derives. These figures therefore set an approximate younger limit for the age of Member 2 in which the polarity change occurs.

With these limits, the upwards transition from reversed to normal polarity low in Member 2 must have an age of 3.32, 2.94 or 2.80 Myr if we use Cox's[12] polarity time scale. The next closest Transitions in the same sense are at 3.90 Myr (Cochiti) and 2.13, 1.98 or 1.79 Myr (Réunion or Olduvai) using the same time scale, and are well outside the limits set by the geomorphological and faunal evidence. It must be emphasised that the palaeomagnetic argument depends heavily upon the limits provided by the other evidence, but if these limits are valid it becomes possible to state that Member 2 of the Makapansgat Formation lies within the Gauss normal epoch and has a maximum age of between 2.8 and 3.3 Myr. The assumed transition low in Member 4 could then be one of those higher up within the Gauss or at its upper boundary. The reversed and intermediate directions high in Member 5 occur after what is believed to be a considerable lapse of time and may be associated either with the Réunion or the Olduvai events.

All of these conclusions depend upon the cleaned palaeomagnetic directions being primary. Full details will be published elsewhere, but the demagnetisation behaviour both in direction and intensity suggests that a primary component has been isolated. Moreover, the mean directions of the normal samples in Member 2 both at sample and at horizon level lie closer to the dipole field than to the present-day field at Makapansgat, which suggests that recent remagnetisation has not occurred.

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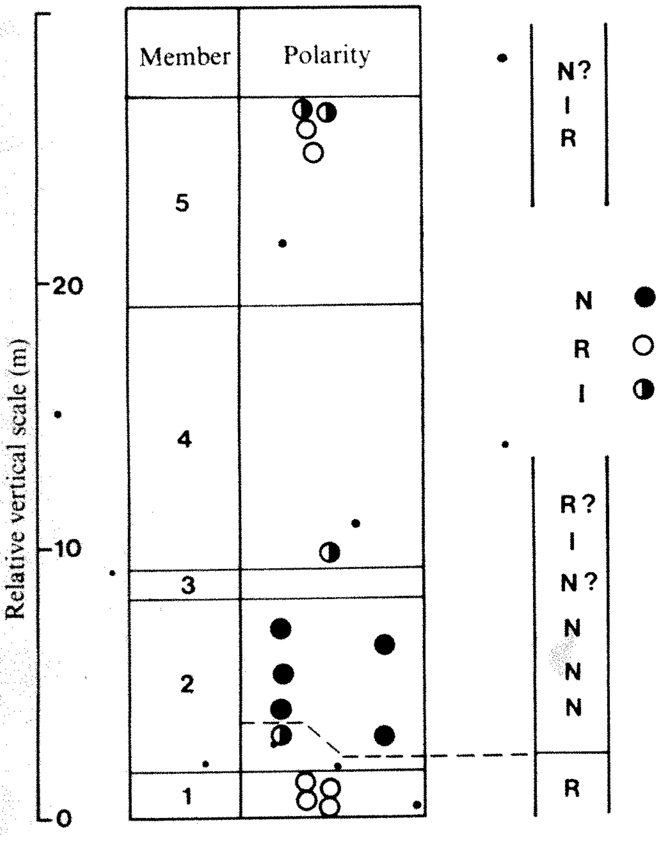
Fig. 1 A schematic stratigraphic column of the Makapansgat Formation showing the polarity at each sampled horizon (see Table 2 for the basic data).



New aspects of Middle Palaeolithic variability in western Europe

OBSERVATIONS are presented here on quantified aspects of Middle Palaeolithic variability which have not been studied systematically so far. The findings relate to differing degrees of secondary modification of lithic artefacts between assemblages. The variations correspond with distinct toolmaking entities defined for early Würm horizons in western Europe. They offer new perspectives, however, on the interpretation of Middle Palaeolithic inter-assemblage variability by suggesting the possible importance of non-cultural causes for some of this variability.

Distinctive Middle Palaeolithic assemblages are found within stratified sequences or between individual sites. These differences have been expressed quantitatively by comparing lithic assemblages through the classificatory method developed by Bordes *et al.*, particularly the polymodal frequency distribution of the racloir index, to which correspond characteristic tool type clusters[1]. Two main industry groups have been defined: le Moustier, which consists of the Denticulate, the Acheulian Tradition (or MTA) types A and B and the Typical Mousterian; and the Charentian, including the Ferrassie and Quina. This classificatory arrangement is the most widely accepted at present.

Controversy persists, however, regarding the significance of 'polytypic' Middle Palaeolithic phenomena. Resolution is theoretically important because of the increased knowledge which may be obtained on the behaviour of Neanderthal populations. Bordes interprets the different industry types as the manifestation of separate toolmaking traditions manufactured by distinct communities often coexisting within a same area[2]. Mellars supports the conclusion that most of the traditions are patterned chronologically[3], while Binford favours a functional interpretation: the different tool type clusters are specialised toolkits for the performance of various tasks by culturally similar populations[4].

The evidence discussed here is derived from some 120 assemblages excavated and sorted typologically by well-known workers over the last 25 yr. The method used concentrates on variable features of broad artefact groups and relies on the Bordes classificatory system. The basic observation unit includes all regularly retouched tools (or implements)—all racloirs types, denticulates and notches, bifaces and types nos 6–8, 30–35, 40–41 and 53–60 of the Bordes type list—whose frequencies are calculated against the number of implements plus all irregularly retouched, utilised or blank Levallois and non-Levallois flakes and blades. A distinction is therefore made between regularly transformed and utilised tools and flakes casually modified or utilised or without macroscopic wear traces. Sub-categories are defined, including (1) 'blanks and utilised flakes', nos 1–5 and 45–50, of the list; (2) non-Levallois unretouched flakes; (3) 'Levallois implements', or the number of pieces among Levallois flakes which are modified into implements; (4) racloirs and (5) denticulates and notches. Ratios of flake tools and blanks to cores per assemblages are also included.

Figure 1 shows the mean and range of implement frequencies for each Middle Palaeolithic industry type. The observed separation into two clusters agrees with a sorting of assemblages into le Moustier and Charentian groups. A maximum distance is found between the Denticulate and Charentian industries and the plotted ranges are discontinuous. The Typical and MTA show intermediary values overlapping with other industry types. A similar pattern was noted among individual sites sequences, for example, at Combe-Grenal, Hauteroche, Caminade, Armand Chadourne and le Maras, suggesting the presence of a recurrent, consistent trend.

Table 1*a* compares averages of frequency variations for artefact sub-categories among Middle Palaeolithic industries. The differential patterns are consistent with those shown for implement frequencies. Values for Levallois implements, though generally higher, conform with previously observed tendencies. In Table 1*b*, ratios of flakes and blanks to cores again conform with the earlier findings, the highest values being associated with the Charentian.

The data suggest that differential blank production and secondary modification are correlated. Higher blanks and flake implements to core ratios may be due to more exhaustive flaking of flint nodules, although other factors may be involved. Taken together, both could be attributed to non-cultural causes, for example, the influence of environmental circumstances inducing more economical uses of lithic material, as illustrated by the following studies.

In one case, it was shown that scarcity of flint in some crystalline regions determined increased secondary flaking and the development of primary flaking techniques making optimal use of available flints[5]. Another study has contrasted within the same le Moustier traditions, the open-air summer camps of short duration with the more sedentary rockshelters or caves settlements. In the latter, more Levallois blank pieces were secondarily transformed into implements than in the former.[6]

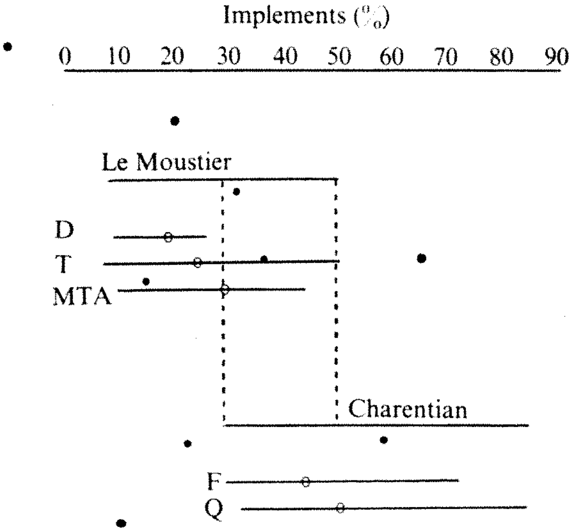


Fig. 1 Average implement frequencies and ranges for Middle Palaeolithic industries. D, Denticulate; F, Ferrassie; MTA, Moustérian de Tradition Acheuléene; Q, Quina; T, Typical; I, implement frequencies. Data are derived from artefact counts of sites in western Europe: Grainfollet (shelter, beach), Méru, Oissel, Houpeville (séries rousse et claire), Bihorel (III), Hamel (8), Busigny, Laigny (C), Evreux (6), Gros-Monts (2), Goderville (s. lustrée) Tillet (s. café-au-lait), Marcoing, Oldbury, Combe-Grenal (1, 6–38, 40–43, 46, 50–52, 54), Pech de l'Aze, locus I (3, 4F, A, B, C, 5, 6, 7), locus II (3, 4B, 4F, 4C2), Hauteroche (1, 3), Bouheben (1, 1′), Nantet I, Abri Chadourne (A, B, C, D), Caminade (1, 2, 3, 3s), la Rochette (7), Las Pénélos, Mas-Viel, Vauzelle, Plateau Baillard (1, 2), Chinchon, le Dau, l'Ermitage, Petit-Puymoyen (Grotte, 2, bréche, 3), Baume-Vallée, le Madriat, St. Maurice-sur-Loire, le Maras (1, 3, 4, 5), Ranc-Pointu, le Figuier, Blanzy, l'Hortus (16–21A, 18–25, 7–17), Rigabe (G), Bas-Guillotte, Grotte Tournal (1, 2, 4, 5), Abri Romani (4–8, 9–9a, 10–13), San Francesco, Kokkinopilos (Greece).

These explanations, though probably correct in their context, do not apply here for various reasons. First, most of the sites represented by the data are from regions where flint or equivalent raw materials are regularly available. Second, the most pronounced quantitative differences involve industries such as the Denticulate and the Charentian which occur mostly in rockshelter or cave sites in Western Europe, often within the same stratified successions. And third, more significantly, average differences in primary and secondary flaking co-vary with defined Middle Palaeolithic traditions, instead of forming non-cultural facies within them. These differences apply to Levallois and non-Levallois flakes. Consequently, the variations do not seem to be related to environmental circumstances such as a settlement dichotomy or regional scarcity of flint.

Table 1 Comparisons of *a*, major artefact class averages and *b*, ratios of flakes and flake tools to cores for each Middle Palaeolithic industry

	a				*b*
Middle Palaeolithic industries	Implements	Utilised and blanks	Levallois implements	Non-Levallois plain flakes	Flakes and flake tools ratios
D	17.1	24.7	35.0	57.7	20.2
T	22.7	26.5	36.4	50.8	22.5
MTA	25.6	25.4	49.4	48.5	14.7
F	43.0	21.4	49.6	35.4	31.0
Q	49.8	10.6	58.6	39.5	33.3
Sample averages	29.9	22.0	43.3	47.9	—
N assemblages	120	120	98	120	94

On the other hand, it would be difficult to test independently a hypothesis stating that these new observations constitute an additional set of cultural norms from distinct toolmaking traditions. Other quantitative data give little support for a 'cultural' hypothesis.

First, the implements frequency histogram for the entire sample is continuous and unimodal, suggesting a single phenomenon, whereas a polymodal model, analogous to that for the racloirs index, would be expected for distinct toolmaking norms. Second, the scatter diagram (Fig. 2) shows that high implement frequencies depend mostly on increased racloirs frequencies. Racloirs along with denticulates and notches are the most frequent Middle Palaeolithic implement classes. Their relative variations provide diagnostic criteria for the identification of most early Würm traditions in the Bordes system. Racloirs predominate strongly in the Charentian, denticulates and notches in the Denticulate and type B Acheulian Tradition Mousterian. We show above that the variations in implement frequencies revealed by this study are due to increases in racloirs manufacture rather than in miscellaneous implement forms. If preference for racloirs expresses the design norms of Charentian populations, it should be emphasised that the manufacture of stylistically alternative forms is never commensurate with that of racloirs in other toolmaking traditions. This analysis is the first to measure this non-equivalence or quantitative asymmetry. It confirms but amplifies the conclusion that racloirs constitute the most dynamic artefactual element in Middle Palaeolithic assemblages and also suggests that more than cultural preferences may be involved in high racloirs frequencies.

The discovery of new dimensions of variability adds to the number of open problems identified with the Middle Palaeolithic. These problems are present at a descriptive (statistical) and interpretative (anthropological) level: (1) to what extent do the graphic patterns for lithic assemblages represent either one or more original populations? (2) The issue of whether inter-assemblage differences can be interpreted in terms of the existence of distinct identity-conscious groups or of several discrete activities within a single one remains unresolved. Both interpretations are theoretically reasonable but neither has been subjected to sufficiently stringent tests.

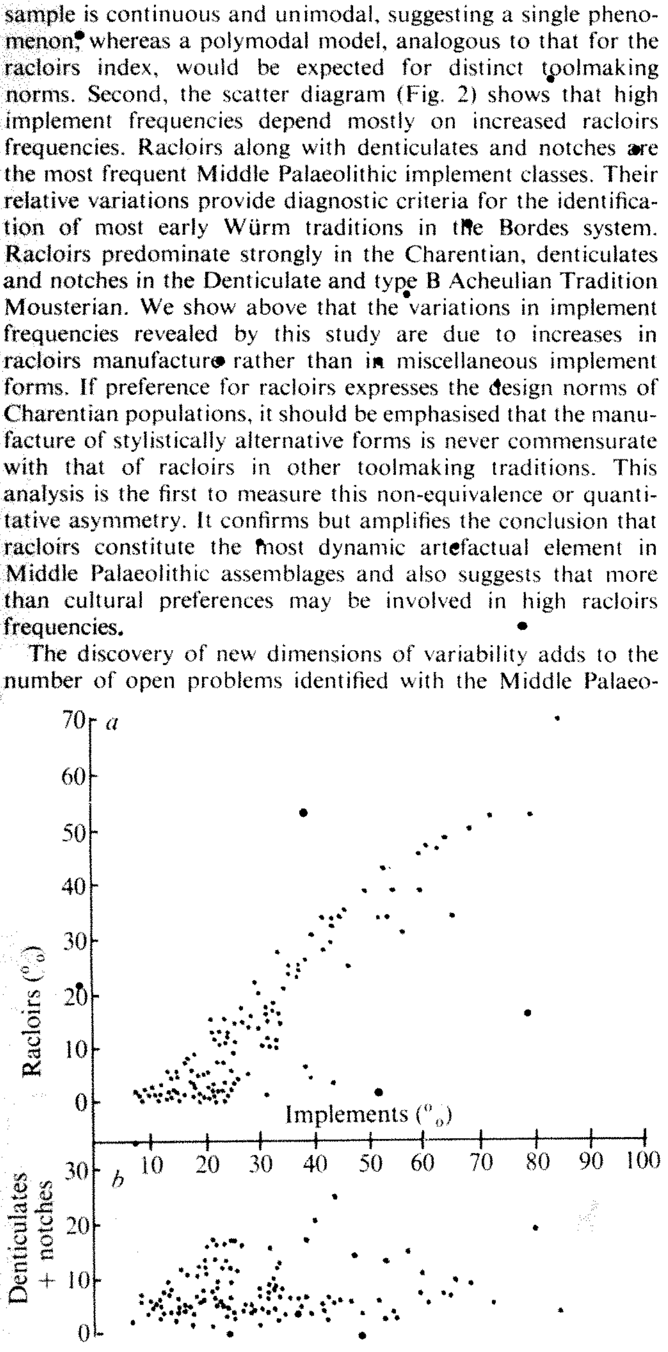


Fig. 2 Scatter diagram of implement frequencies (I) with *a* racloirs (R) frequencies and *b* denticulates and notches (D + N) frequencies. Only *a* shows positive association.

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'Modern' lizard from the Upper Triassic of China

LIZARDS are the most numerous, varied and widespread of living reptiles. An essentially modern lizard fauna has existed throughout the Tertiary and members of many modern lizard families are known from the late Cretaceous. Earlier in the Mesozoic, very few fossils of lizards are known. Lizards from the Upper Jurassic may be related to the ancestors of modern groups, but are placed in distinct families. Lizards previously described from the Upper Triassic of Europe[1] and North America[2] are considerably more primitive, retaining many cranial features in common with their ancestors from the Upper Permian and Lower Triassic[3]. We give here the first description of an Upper Triassic lizard with an essentially modern cranial morphology. This specimen is interesting with respect to the origin, differentiation and zoogeography of the modern lizard groups.

Class Reptilia
Order Squamata
Suborder Lacertilia
Infraorder Incertae Sedis
Family Fulengidae n.
Fulengia youngi, gen. nov. et. sp. nov.

Holotype: CUP 2037, skull, lower jaws and associated vertebra; collection of the Catholic University of Peking, now housed in the Field Museum of Natural History, Chicago. Collected by Fr E. Oehler, SVD in 1948 or 1949; part of a rich fauna of therapsids and archosaurs[4].

Locality and horizon: Ta Ti, Lufeng Basin, near K'un-ming, Yunnan Province, China. Dark Red Beds, Lower Lufeng series, Upper Triassic, equivalent to the Norian or Rhaetic of Europe[4]. The generic name is an anagram based on the locality from which the specimen was collected; the specific name honours the work in this region by Dr C. C. Young.

Diagnosis: a combination of the following characteristics distinguishes this form from other lizards previously described from the Mesozoic and Cainozoic: median

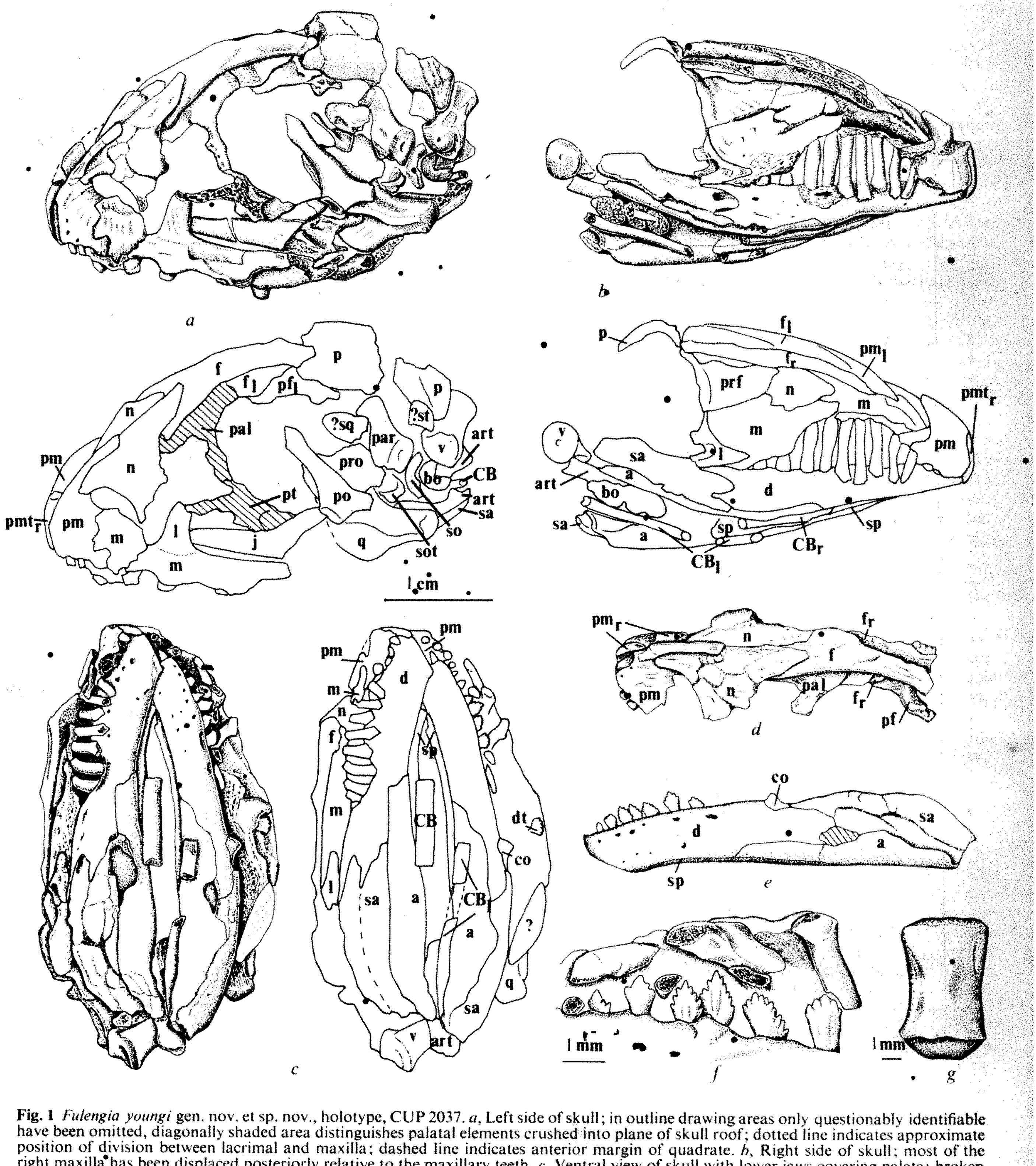

Fig. 1 *Fulengia youngi* gen. nov. et sp. nov., holotype, CUP 2037. *a*, Left side of skull; in outline drawing areas only questionably identifiable have been omitted, diagonally shaded area distinguishes palatal elements crushed into plane of skull roof; dotted line indicates approximate position of division between lacrimal and maxilla; dashed line indicates anterior margin of quadrate. *b*, Right side of skull; most of the right maxilla has been displaced posteriorly relative to the maxillary teeth. *c*, Ventral view of skull with lower jaws covering palate; broken line on surangular indicates normal dorsal extent of displaced angular. *d*, Dorsal view of anterior portion of skull. *e*, Lateral view of left lower jaw. *f*, Teeth of left dentary (below) and maxilla; teeth seen in section are from the more anterior portion of the maxilla that has been telescoped between the other elements. *g*, Associated vertebra in ventral view presumed anterior end uppermost. (*a–e*, ×2; *f*, ×8; *g*, ×4). a, Angular; art, articular; bo, basioccipital; CB, first certaobranchial; co, coronoid; d, dentary; dt, displaced tooth; f, frontal; j, jugal; l, lacrimal; m, maxilla; n, nasal; p, parietal; pal, palatine; par, paroccipital process; pf, postfrontal; pm, premaxilla; pmt, premaxillary tooth; po, postorbital; pro, proötic; pt, pterygoid; q, quadrate; sa, surangular; so, supraoccipital; sot, spheno-occipital tubercle; sp, splenial; sq, squamosal; st, supratemporal; v, vertebra; subscripts l and r refer to bones from left and right side where there is a possibility of confusion. In the case of the frontal, they indicate broken elements of a median bone.

frontal; paired premaxillae with long nasal processes; large upper temporal opening with a margin partially formed by the postfrontal; postorbital triradiate and nearly vertical; lateral articular surface of paroccipital process large, indicating support of quadrate and squamosal; lacrimal reduced; approximately five teeth in the premaxilla, nine in the maxilla and 13 in the dentary; teeth fully pleurodont, closely resembling those of the modern genus *Iguana iguana*; laterally compressed, cuspate crowns whose anterior margin is angled toward the midline; low coronoid process, approximately midway between articular and symphysis; splenial extending from behind coronoid to symphysis; vertebrae weakly procoelous; skull of only known specimen 38 mm long. The absence of comparative material from the early Mesozoic makes composition of a proper differential diagnosis impossible. There is no clear basis for determining which characters may be diagnostic at the level of species, genus or family, although it is probable that dental characteristics and relatively large size are typical of the species or genus, but not family. In spite of the probable exclusion of this genus from previously recognised lizard infraorders, we do not consider it desirable to create a new infraorder on the basis of a single incomplete skull.

The skull (Fig. 1) is flattened laterally and telescoped from back to front, as well as having some individual elements further displaced. Simmons[4] indicates that many specimens from this locality give the impression of being coprolitic; passage through the digestive system may account for the damage to this specimen. Although the bones identified in Fig. 1 are for the most part clearly defined, in other areas of the specimen it is not possible to differentiate bone from matrix. Some of the bones are broken and deeply fissured, but there is little difficulty in differentiating sutures from breaks.

The configuration of most of the bones of the roof of the skull can be seen from one side or the other. Important exceptions are the parietal and squamosal. The area of the parietal is too poorly preserved to show whether or not the bone is paired, but it appears to be fused medially. The parietal foramen appears to have been located at the juncture between the frontal and parietal. The position of the paroccipital process defines the extent and orientation of the squamosal, but the specific relationship with the quadrate (the dorsal portion of which is covered by the proötic and postorbital) cannot be determined.

With the exception of brief reference to more advanced forms[1], our knowledge of Late Triassic lizards is limited to the specialised gliding forms from Great Britain and North America in the family Kuehneosauridae. In spite of their extreme postcranial specialisation, these genera retain a skull morphology similar to that of the late Permian and early Triassic Paliguanidae[3] from South Africa. The oldest described members of the infraorders Gekkota, Iguania, Scincomorpha and Anguimorpha are from the late Jurassic of Europe and China[5]. The only living infraorder of lizards not recognised in the Upper Jurassic is the Amphisbaenia, known only since the early Tertiary[6]. Differentiation of the modern infraorders presumably began early in the Jurassic or even in the Upper Triassic.

Fulengia does not show the particular combination of features by which members of the Gekkota, Anguimorpha or Amphisbaenia are recognised. What is known of the skull, as restored (Fig. 2), resembles in a general way the pattern of primitive members of the Iguanidae, some large teiids and members of the extinct scincomorph families recently described from the Upper Cretaceous of North America and Mongolia[7]. Most of the features that it shares with these groups, however, are clearly primitive; for example, the presence of a large dorsal temporal opening, a complete postorbital bar and a vertically oriented, triradiate postorbital, and do not support assignment to any of these families. The massive lower jaw and the tiny coronoid distinguish this genus from members of all living reptile infraorders.

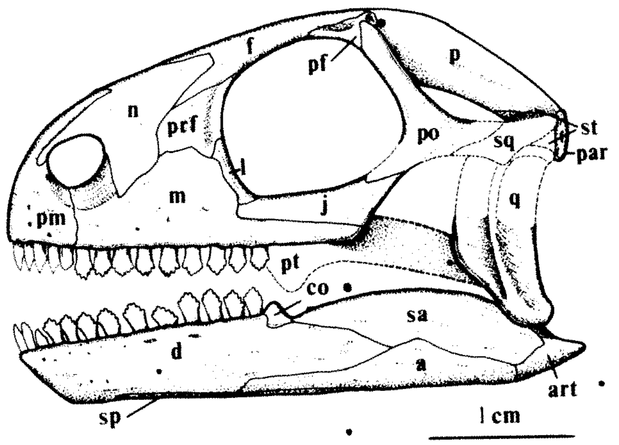

Fig. 2 *Fulengia youngi*, gen. nov. et sp. nov., reconstruction of the skull in lateral view ×2. Bones and teeth shown in broken lines are either missing or covered by other elements in the specimen. They are reconstructed on the pattern of the living *Iguana*. Abbreviations as in Fig. 1.

The dentition of *Fulengia* (Fig. 1*f*) is presumably a specialised characteristic and probably less important than the pattern of the skull bones in establishing infraordinal relationships. Multicuspate, laterally compressed tooth crowns similar to those of *Fulengia* are known in numerous living lizards belonging to diverse taxonomic groups. Where stomach contents have been studied[8], such a pattern is associated with a primarily herbivorous diet. Accepting a skull : trunk ratio similar to that of living iguanids with similar skull size[9] and length : weight ratios determined by Pough[10], the weight of this specimen would be approximately 80 g—towards the top of the range for primitive insectivores, but near the bottom of the range for herbivores[10]. Dental specialisation toward a herbivorous diet and relatively large size are features not expected in primitive lizards[10] and particularly not in the group(s) ancestral to the advanced infraorders, most of which are represented by small, apparently insectivorous, forms in the late Jurassic. The relatively large size of the skull was probably an important factor in its preservation, in view of the extremely poor fossil record of other, smaller, lizards in the early Mesozoic.

Fulengia itself can be viewed as a precociously specialised genus, divergent from the main line of lizard evolution. Other members of the family to which it belonged may have been closer to the ancestry of modern groups. The Fulengidae may (1) be related to the stock from which the known iguanians arose; (2) represent an earlier stage, close to the ancestry of most lizard infraorders, or (3) exemplify an independent achievement of some modern lizard features from the primitive eolacertilian stock. The absence of comparable forms in the Triassic and early Jurassic makes it difficult to establish the relationship of *Fulengia* more specifically.

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Adaptive reduction in permeability of avian eggshells to water vapour at high altitudes

We report here the existence of an altitudinal cline in permeability of eggshells to water vapour for eggs of a North American species of bird. This pattern of geographic variation in permeability of eggshells seems to have arisen as an adaptive response to altitudinal variation in the diffusivity of water vapour through air. Diffusivity of atmospheric gases has not previously been widely recognised as an important component of the physical environment to which organisms are exposed, and our findings provide further evidence for the pervasiveness of natural selection.

A bird egg loses water continuously during normal incubation owing to the outward diffusion of water vapour through pores in the calcareous shell[1–3]. The loss of a certain amount of water from the egg is essential for the normal development and hatching of the embryo[4], for the escape of water allows an air space to form in the blunt end of the egg[5] and this space is the source of air for the first breaths taken by the embryo before hatching[1]. Reserves of water within the egg to support the metabolism of the developing embryo are fixed in amount, however, and the loss of large quantities of water from the egg could be detrimental[6]. Thus, porosity of the eggshell is delicately adjusted (in an evolutionary sense) to assure that only as much water is lost from the egg during incubation as is required for formation of an air space of the necessary volume[3,7].

Eggs laid by birds breeding at high altitudes face a potential disruption of the balance between water loss and water conservation[3], simply because molecules of water vapour diffuse more rapidly through air in conditions of low atmospheric pressure characterising high altitude environments than in atmospheric conditions of low altitudes[8]. Therefore, as a working hypothesis, we predicted that permeability of eggshells to water vapour is lower in eggs of birds breeding at high altitudes than in eggs of conspecifics breeding at low altitudes, thereby compensating for the increased diffusivity of water vapour in air at high altitudes.

During June 1976, eggs of barn swallows (*Hirundo rustica*) were collected from nests at nine localities along an altitudinal gradient running from the town of Crook in the South Platte River Valley of northeastern Colorado (elevation 1132 m), to South Park in the mountains of central Colorado (elevation 2883 m). The diffusivity of water vapour in air is approximately 25% higher at South Park than at low elevations in northeastern Colorado. Only one egg was taken from each nest because eggs of a single female are likely to have similar permeabilities to water vapour[9].

Following transport of the eggs to our laboratory at Colorado State University, the eggs were placed randomly in vented desiccators above flakes of potassium hydroxide to maintain a dry atmosphere[1]. The desiccators were held in a constant temperature cabinet at 25 °C, a temperature too low for embryogenesis to proceed[10]. Each egg was weighed six times on an analytical balance at approximately 24-h intervals, and the rate of weight loss—which we attribute largely to the escape of water vapour—was computed by a linear regression procedure. The volume of each egg was then measured by displacement of water, and its surface area calculated using the equation of Paganelli, Olszowka and Ar[11].

Assuming that the vapour pressure at inner surfaces of eggshells was 23.76 torr (that is, the vapour pressure of pure water at 25 °C)[12,13], the conductance of eggs to water vapour was calculated from the formula

$$G_{H_2O} = M_{H_2O}/\Delta P_{H_2O}$$

where G_{H_2O} is the conductance of an egg to water vapour (μg d^{-1} torr^{-1}), M_{H_2O} is the rate of water loss (μg d^{-1}), and ΔP_{H_2O} is the gradient in vapour pressure across the eggshell (torr). All values were corrected to sea-level pressure (ref. 8).

A regression analysis of variance (Table 1) revealed a linear decline in conductance of barn swallow eggs to water vapour with increasing altitude of the nesting site (Fig. 1). This reduction in water-vapour conductance of swallow eggs from montane localities is similar to that reported earlier for eggs of domestic fowl maintained for several generations at a high altitude[14], but differs from the latter in an important respect. Reduced conductance of eggs of domestic fowl at high altitude just compensates for the increased diffusivity of water vapour, resulting in total water losses from eggs during incubation that are similar to those experienced by eggs from low altitudes[14]. In contrast, the adaptive reduction in conductance of swallow eggs with increasing altitude of the nesting site is greater than that required simply to compensate for the increased diffusivity of water vapour at high altitude. For example, whereas a 17% reduction in conductance of swallow eggs would just compensate for the increased diffusivity of water vapour at 3000 m as compared to 1500 m, the observed reduction in conductance between these elevations is 59%.

Table 1 Regression analysis of variance of conductances of barn swallow eggs to water vapour in relation to altitude of the nesting sites

Source of variation	Sum of squares	d.f.	Mean squares	*F*-ratio	*P*
Linear regression	183,990	1	183,990	13.07	<0.005
Deviations from linearity	114,049	7	16,293	1.16	>0.25
Error	351,886	25	14,075		
Total	649,925	33			

This analysis indicates that a linear model is adequate to describe geographic variation in conductances of eggs to water vapour, and that the slope of the line is significantly different from zero.

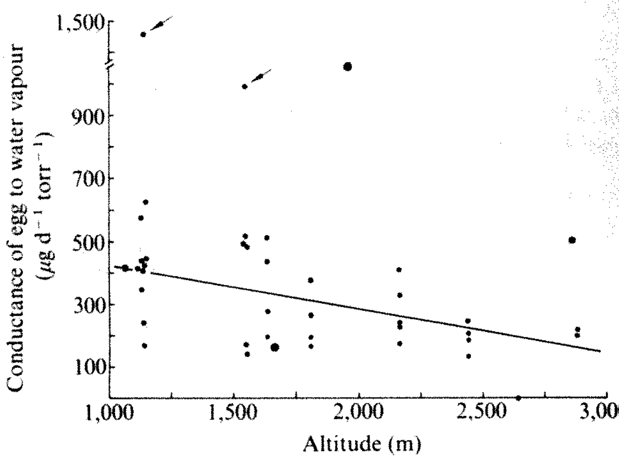

Fig. 1 Linear regression of conductances of barn swallow eggs to water vapour (corrected to sea-level pressure) against altitude of the nesting sites ($y = 564 - 0.140x$). The two extraordinarily high values identified with arrows represent eggs that probably suffered hair-line cracks during transport, and these values were not included in the analysis of variance (Table 1). Bartlett's test indicates that we are justified in assuming homogeneity of sample variances ($\chi^2 = 9.65$; d.f. = 8; $P > 0.1$). The correlation coefficient for the regression line is 0.53.

Adaptation of barn swallows to high altitudes differs from

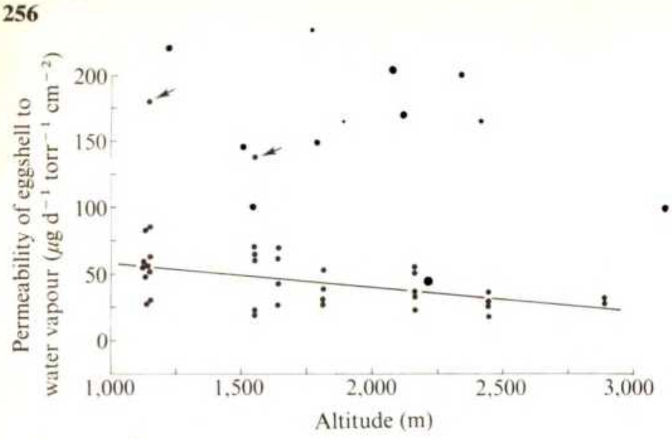

Fig. 2 Linear regression of permeabilities of barn swallow eggshells to water vapour against altitude of the nesting sites ($y=75.18-0.0176x$). Permeability of each eggshell was computed by dividing the conductance of the egg to water vapour by the calculated value for surface area[1]. The slope of the line is significantly different from zero ($F_{1,25}=11.67$; $P<0.005$), and the data do not depart significantly from linearity ($F_{7,25}=1.39$; $P\approx 0.25$). The correlation coefficient for the regression line is 0.50. Arrows identify the statistical outliers to which reference was made in the caption to Fig. 1.

the example of domestic fowl in another important way. Adaptive reduction in conductance of hens' eggs at high altitude stems from reductions both in size of eggs (and therefore in surface area) and in permeability of eggshells to water vapour[14]. In contrast, eggs of barn swallows do not vary in size among the populations sampled in this investigation ($F_{8,27}=1.49$; $P>0.1$), and the entire decline in conductance with increasing elevation is attributable to a parallel decline in permeability of eggshells (Fig. 2). Thus, domestic fowl and barn swallows exhibit different adaptive strategies in their responses to reduced atmospheric pressure.

The observed pattern of altitudinal variation in permeability of barn swallow eggshells to water vapour has important implications pertaining to the comparative biology of montane and piedmont populations of the species. For instance, eggs of various bird species experience a loss of water during incubation amounting to 14–18% of the weight at laying[3,7]. Assuming that eggs of barn swallows from montane and piedmont populations experience similar fractional weight losses prior to hatching, then incubation periods may be longer at high altitudes[3,14], or effective ventilation of nests may be increased at montane localities, thereby providing for lower vapour pressures in air adjacent to incubating eggs and for higher rates of water loss from eggs during natural incubation[7].

The porosity of avian eggshells apparently is a heritable characteristic[15]. The size and number of pores through an eggshell is established at the time of shell formation[9], and the permeability of the eggshell to water vapour remains constant throughout incubation[16]. When heritability of porosity is combined with a tendency for birds to return to the locality where they were born[17], it is possible for natural selection to act on variation in porosity of eggshells to effect a differentiation of populations with respect to this characteristic[18]. Presumably, this explanation accounts for the origin and maintenance of the clinal pattern of variation observed by us for eggs of barn swallows.

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Virus-like particles in the nematode *Romanomermis culicivorax* (Mermithidae)

EARLY reports of virus infections of nematodes remain unconfirmed[1,2], although more recently virus-like particles were reported occurring in the intestinal cells of a plant-parasitic nematode[3]. The only confirmed reports of viruses found inside nematodes are plant viruses which occur in the digestive tracts of their nematode vectors[4]. We report here the presence of virus-like particles in cells of the infective stages of the mosquito parasitic nematode, *Romanomermis culicivorax*.

Cultures of *R. culicivorax* Ross and Smith (*Reesimermis nielseni* Tsai and Grundmann) had been cultured *in vivo* in larvae of the mosquito *Culex pipiens quinquefasciatus* Say.

Cultures containing eggs of the nematode were flooded with water and yielded preparasitic (infective stage) juveniles that were prefixed for 1 h in phosphate-buffered 1% gluteraldehyde then transferred to a 1% solution of osmium tetroxide. They were then placed in agar blocks, dehydrated in an ethanol series and double embedded in Araldite. Sections made on a Porter–Blum microtome were stained with uranyl acetate and examined with a Philips EM 300 electron microscope.

Each of 10 preparasitic juveniles of *R. culicivorax* examined contained virus-like particles in the hypodermal cords, cells

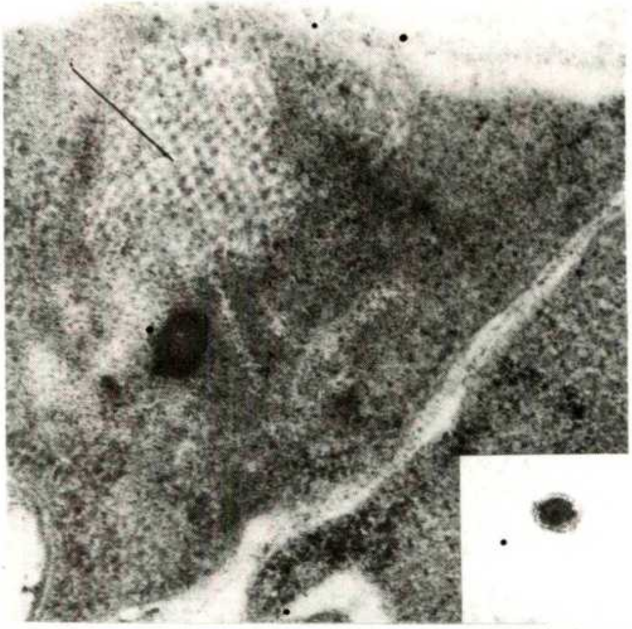

Fig. 1 Crystalline array of virus-like particles (arrow) in hypodermal cord of preparasitic juvenile of *R. culicivorax* (× 50,000). Insert shows isolated virus-like particle with inner core and outer envelope (× 100,000).

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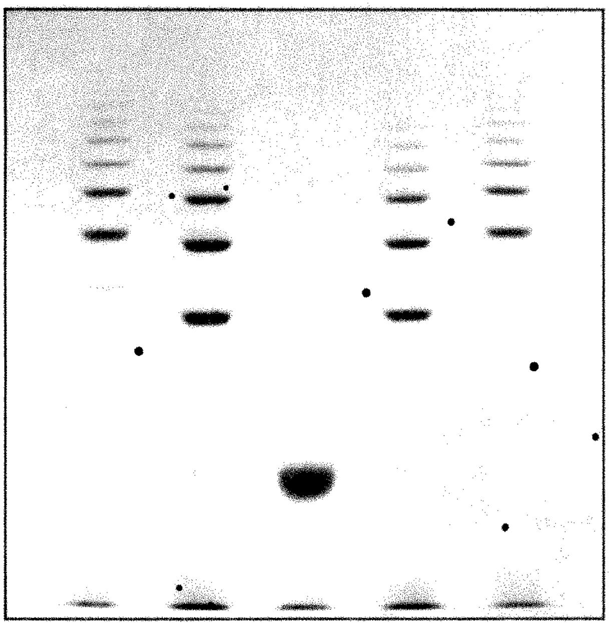

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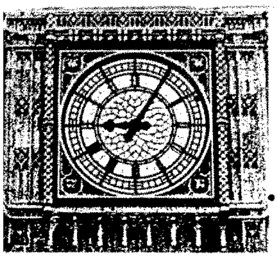

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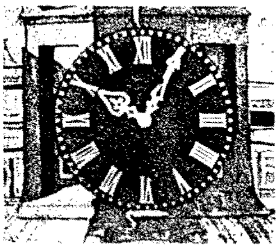
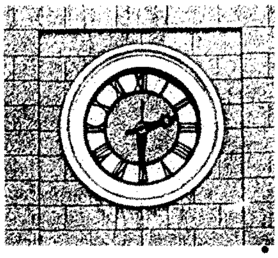
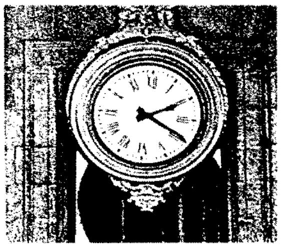


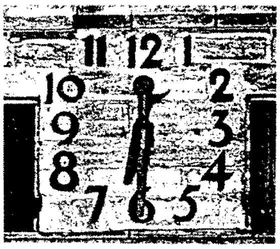
Amsterdam. Every day at 10.05 hours, the clock at the Mint Tower indicates the departure of a Swissair plane for Switzerland. And also at 16.45, 20.15, and 21.05.



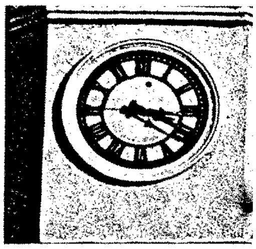
Athens. On Wednesdays and Sundays at 14.30 hours, the clock at the Mitropolis Cathedral indicates the departure of a Swissair plane for Switzerland. And also eleven times every week at different hours.



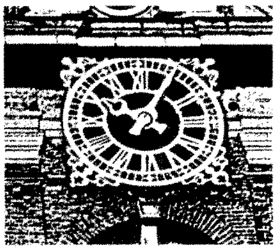
Barcelona. Every day at 14.20 hours, the clock at the Town Hall indicates the departure of a Swissair plane for Switzerland. And also five times every week at 15.30.



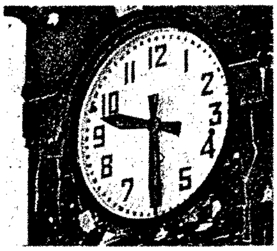
Basel. Eighty-eight times every week the clock at the Spalentor indicates the departure of a Swissair plane for 11 destinations.



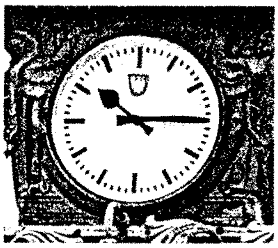
Belgrade. Every day at 15.20 hours, the Sahat-Kula Clock Tower of Kalemegdan Fortress indicates the departure of a Swissair plane for Switzerland.



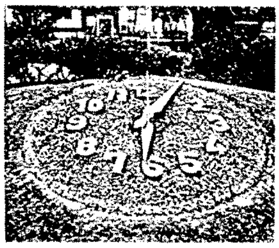
Copenhagen. Every day at 10.05 hours, the clock of Our Saviour's Church indicates the departure of a Swissair plane for Switzerland. And also at 12.15, and 18.55.



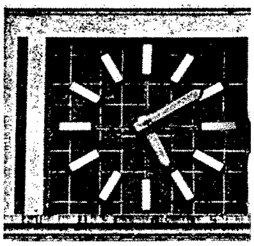
Düsseldorf. Every day at 09.30 hours, the Water Gauge Clock indicates the departure of a Swissair plane for Switzerland. And also at 13.20, 16.20, and 20.05.



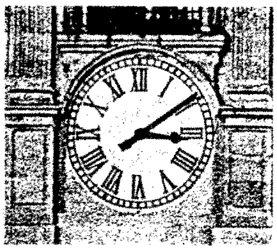
Frankfurt. Every day at 10.15 hours, the Little Clock Tower indicates the departure of a Swissair plane for Switzerland. And also at 16.25, 20.50, and 21.00.



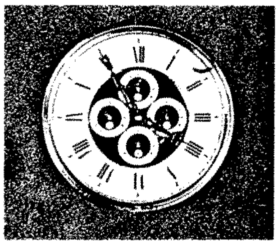
Geneva. Three hundred and one times every week, the Flower Clock indicates the departure of a Swissair plane for 61 destinations.



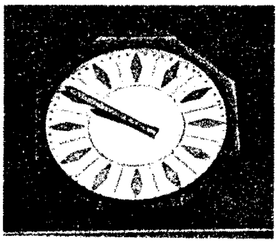
Genoa. Five times every week at 17.10 hours, the clock of the Grattacielo indicates the departure of a Swissair plane for Switzerland.



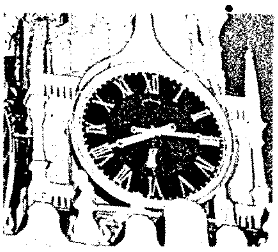
Malaga. Every Monday, Friday, Saturday and Sunday at 15.10 hours, the clock of the Cathedral indicates the departure of a Swissair plane for Switzerland.



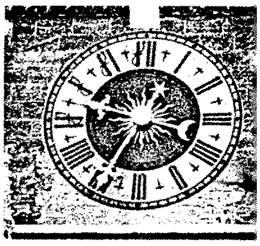
Marseilles. Every day at 15.55 hours, the clock in the Palais de la Bourse indicates the departure of a Swissair plane for Switzerland.



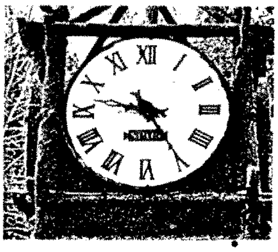
Milan. Every day at 09.50 hours, the clock at the Central Station indicates the departure of a Swissair plane for Switzerland. And also at 13.10, and 16.35.



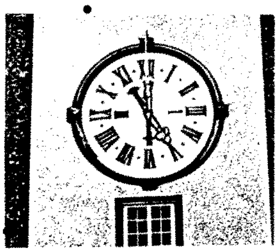
Moscow. Every Tuesday, Wednesday and Friday, at 08.15 hours, the Kremlin Tower Clock indicates the departure of a Swissair plane for Switzerland.



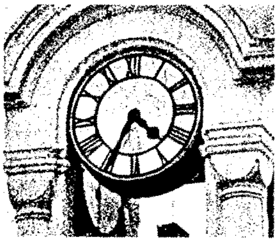
Munich. Every day at 09.35 hou the clock at the Town Hall indicates the departure of a Swissair plane for Switzerland. And also at 13.10, and 20.20.



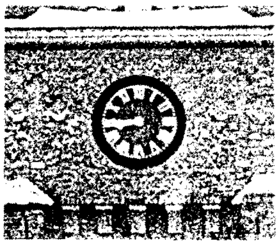
Rome. Every day at 09.25 hours, the Waterclock at Villa Borghese indicates the departure of a Swissair plane for Switzerland. And also at 14.30, and 20.15.



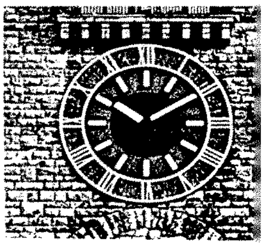
Salzburg. Every Monday, Wednesday, Friday and Sunday at 17.00 hours, the clock at the Town Hall indicates the departure of a Swissair plane for Switzerland.



Sofia. Starting April 1, 1977, on Tuesdays and Fridays at 16.35 hours, the clock at the Central Halls indicates the departure of a Swissair plane for Switzerland.



Stockholm. Every day at 08.45 hours, the clock of the Riddarholms Church indicates the departure of a Swissair plane for Switzerland.



Stuttgart. Every day at 10.10 hours, the clock at the Central Station indicates the departure of a Swissair plane for Switzerland. And also at 13.35, and 20.30.

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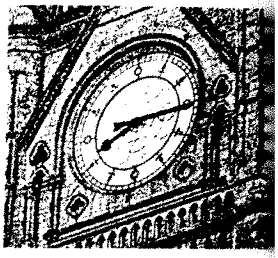
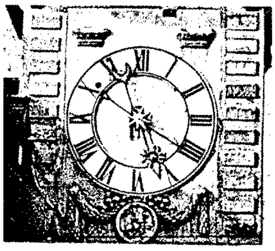
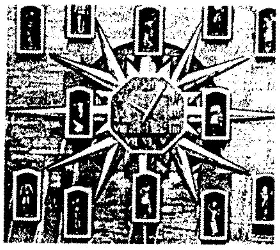


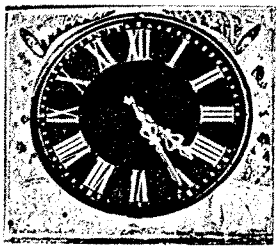
Manchester. Every day at 08.15 hours, the Town Hall Clock indicates the departure of a Swissair plane to Switzerland – with world-wide connections.



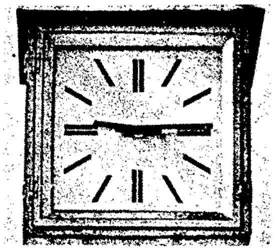
Berne. Every day at 05.20 hours, the Clock Tower indicates the departure of a Swissair bus for Zurich. And also at 09.00, 10.00, 12.00, 15.50, 17.00, and 19.30.



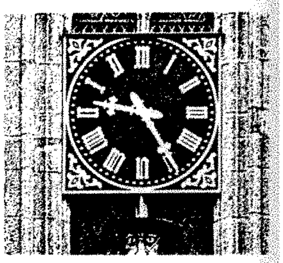
Brussels. Every day at 10.05 hours, the clock of Mont des Arts indicates the departure of a Swissair plane for Switzerland. And also at 15.50, 20.20, and 21.05.



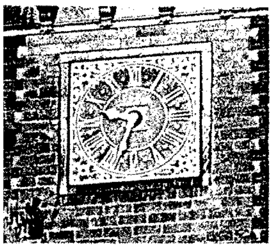
Bucharest. Every Monday, Wednesday and Friday at 16.25 hours, the clock at the Ministry of Agriculture in the centre of Bucharest indicates the departure of a Swissair plane for Switzerland.



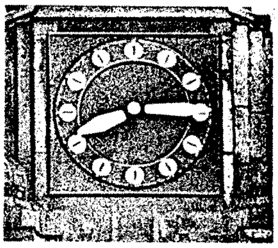
Budapest. Every day at 09.15 hours, the clock at the Pest end of the Margareth Bridge indicates the departure of a Swissair plane for Switzerland.



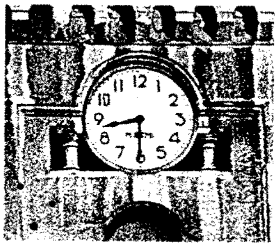
Cologne. Six times every week at 09.25 hours, the clock at the City Hall indicates the departure of a Swissair plane for Switzerland.



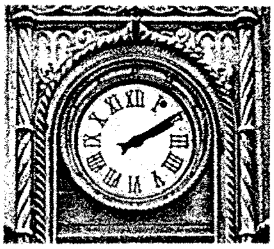
Hamburg. Every day at 09.35 hours, the clock of the Town Hall indicates the departure of a Swissair plane for Switzerland.



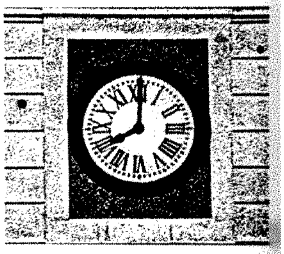
Helsinki. Every day at 08.15 hours, the Clock Tower of the Railway Station indicates the departure of a Swissair plane to Switzerland.



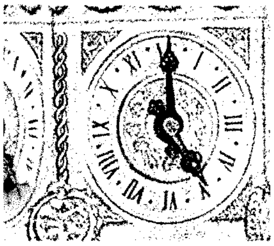
Istanbul. Every day at 08.30 hours, the clock on the garden gate of the University indicates the departure of a Swissair plane for Switzerland. And also every Wednesday and Sunday at 14.45.



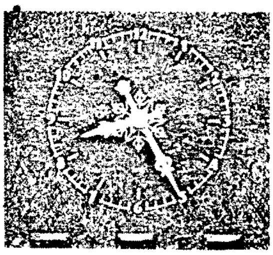
Lisbon. Every day at 14.10 hours, the clock at the Rossio Railway Station indicates the departure of a Swissair plane for Switzerland.



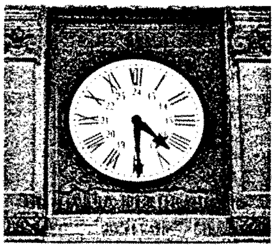
Madrid. Every day at 08.00 hours, the clock of Puerta del Sol indicates the departure of a Swissair plane for Switzerland. And also at 15.50.



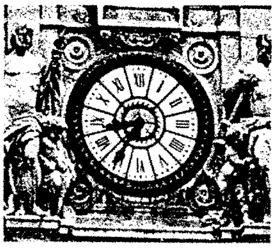
Nice. Every day at 17.00 hours, the clock of Saint-François Tower indicates the departure of a Swissair plane for Switzerland. And also at 19.45.



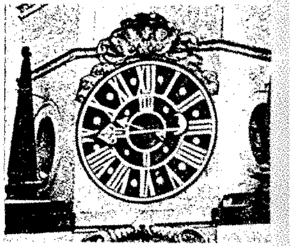
Oslo. Every day at 08.25 hours, the clock of the Town Hall indicates the departure of a Swissair plane for Switzerland.



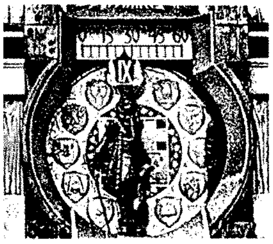
Palma de Mallorca. Every Tuesday, Saturday and Sunday at 16.30 hours, the clock of the Town Hall indicates the departure of a Swissair plane for Switzerland.



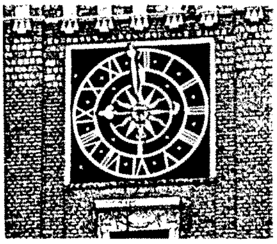
Paris. Every day at 08.35 hours, the clock at the Hôtel de Ville indicates the departure of a Swissair plane for Switzerland. And also at 09.00, 10.15, 11.45, 16.00, 16.20, 17.45, 19.45, 20.35, 21.45, and 21.50.



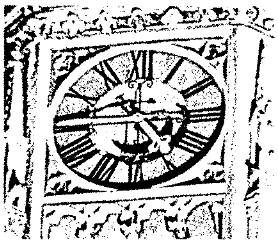
Prague. Five times every week at 09.45 hours, the clock of the Loreta Church indicates the departure of a Swissair plane for Switzerland.



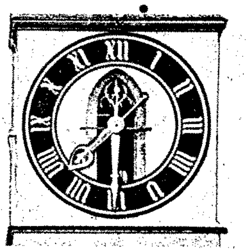
Vienna. Every day at 09.25 hours, the Anker Clock indicates the departure of a Swissair plane for Switzerland. And also at 13.25 and 20.30, and every Friday at 05.10.



Warsaw. Every Tuesday, Wednesday and Friday at 09.00 hours, the clock at the Kings Palais indicates the departure of a Swissair plane for Switzerland.



Zagreb. Every day at 16.45 hours, the clock at the Cathedral of St. Stephen the King indicates the departure of a Swissair plane for Switzerland.



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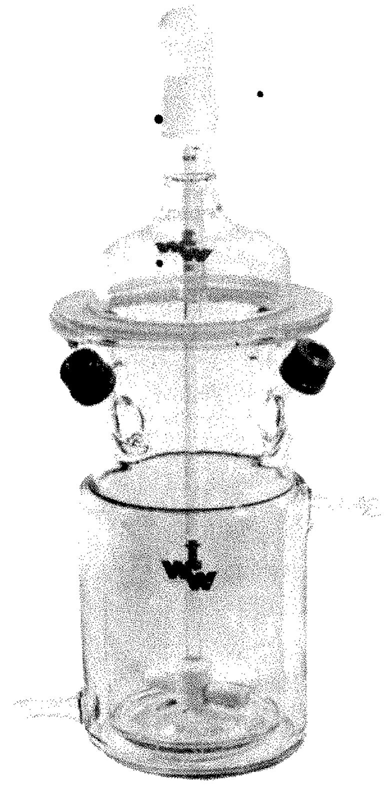
Spinner for Tissue Culture



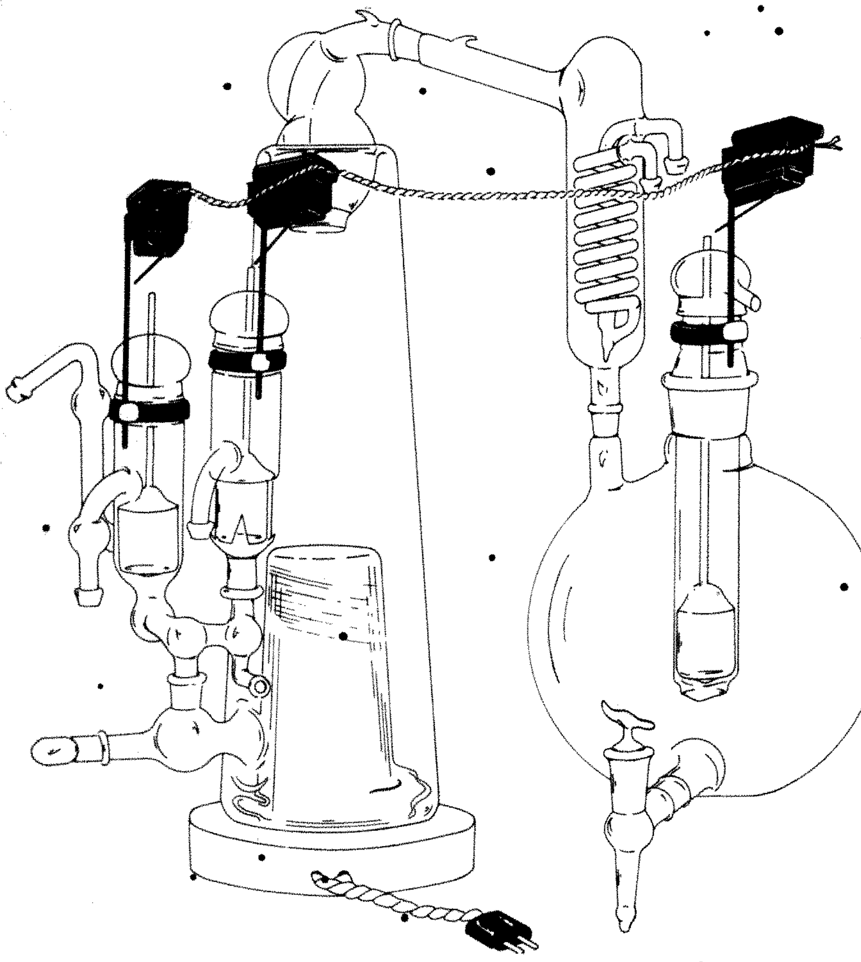
A Water Jacketed Culture Spinner Vessel, the use of which is a very efficient method for the culture of a wide range of cell types that do not need to adhere to a surface for their proliferation. It has been used successfully for the culture of a variety of animal as well as human cells which were derived from normal and malignant tissue. For example, for the culture of cells derived from human osteosarcoma, rhabdomyosarcoma and Rous sarcoma virus transformed cells. In addition it has been used for the culture of human leukaemic cells, lymphoblasts from normal individuals and murine plasmacytoma cells. In each case the cells have had to be established and actively proliferated in vitro before they could be transferred to the spinners, which then have provided the most efficient way for large-volume culture with a very high percentage of viability.

The spinner has a double wall for use as a water jacket for the temperature control of cultures outside an incubator. A water circulator is necessary in this case to give a sufficiently rapid water flow for close temperature control, though the same circulator may serve several spinners. For each spinner a magnetic stirrer is needed to ensure continuous mixing of the cell suspension.

Each spinner can be provided with four openings. For example,

1. an opening for the continuous supply of the appropriate gas mixture into the culture environment. (In order to avoid contamination the gasses should pass through a sterile cotton-plug filter.)
2. an outlet for pressure equalisation. (This, too, should have a sterile cotton-plug filter.)
3. an outlet with a glass tube to the bottom of the vessel for drawing-off quantities of cell suspension under sterile conditions.
4. an opening for the addition of culture fluid (with or without use of a peristaltic pump). This is not essential under normal growth conditions since the culture media can be added through the top opening.

Circle No. 17 on Reader Enquiry Form.



The SCORAH Automatic Electric all glass WATER STILL (patented)

Automatic Can be left on without attention. Heater current cuts off if water supply is interrupted, or inadequate or forgotten. When receiver (5 litres capacity) is full, current cuts off. When distilled water is drawn from receiver, the still resumes operation until receiver is refilled.

Purity Electrostatically charged condenser droplets can pick up fumes, dust and air-borne organisms from the laboratory atmosphere. The design of the receiver vessel ensures absence of such contamination.

Efficiency Heater of Scorah design and manufacture, consumption 1,700 watts, output 2.4 litres per hour (the full theoretical yield). Cost less than 2p per gallon at ½p per unit.

Safety The heating element is out of contact with the feed water, thus eliminating danger from "live" water supply or electrolytic effects of "burn out".

Assembly No retort stands of clamps are required. The 2½-in. diameter ball joint gives a stainless set-up. Outlet cap on boiler for quick periodical drain-off of sediment. The still may be installed on a shelf or platform about 1 ft. above bench level near a sink and enclosed in a transparent plastic cover to keep out dust.

Heat-resisting glass. Interchangeable ground glass joints.

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associated with the amphidial nerves, and spaces of the pseudocoelom. The particles were isometric, developed in the cytoplasm and those found outside the cell were enveloped (Fig. 1 insert). The diameter of the enveloped particles was approximately 50 nm while core (without envelope) measured 33 nm. In the host cells, the particles were either arranged in a crystalline array or randomly in the cell (Fig. 1). In those cases where development occurred in the hypodermal cords, damage to the latter was evident through cell disruption and lysis. Other stages of the nematode have not yet been examined for the presence of similar particles. The virus-like particles could be restricted to this or other nematodes or they could have been originally acquired through contact with mosquito larvae.

If the particles are infective, they would have to be transovarially transmitted in the nematode because the chances that they were acquired during nematode development in the egg or in the short time the preparasitic had emerged from the egg are very slight.

The fact that *R. culicivorax* is being commercially produced for widescale release in gardens and the field clearly amplifies the importance of this discovery. Damage to the hypodermal cords by the virus-like particles could lower the efficiency of this nematode as a biological control agent.

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Nitrogenase activity of endophyte suspensions derived from root nodules of *Alnus, Hippophaë, Shepherdia* and *Myrica* spp.

STUDIES of nitrogenase of root nodule homogenates have focused chiefly on the *Rhizobium*–legume system. Little is known about the nitrogenase of actinomycete-like endophytes of root nodules on woody, non-leguminous plants. Although nitrogen fixation has been reported in suspensions of the endophyte from *Myrica cerifera*[1,2], the results were either of very low significance or irreproducible. In both cases dithionite and oxygen were added, which seems to be contradictory Subsequent attempts to detect nitrogen fixation in non-leguminous nodule homogenates have been unsuccessful and investigations stopped at an early stage. We have found[3], however, that reasonable levels of acetylene reduction by nodule homogenates of *Alnus glutinosa* can be measured if 0.3 M sucrose and 0.1 M dithionite are present during anaerobic homogenisation. The latter was essential because the nodule material contains large amounts of phenolic compounds, which after oxidation inhibit nitrogenase activity. While determining optimal conditions for the nitrogenase assay it was found that the reaction was ATP-dependent but that addition of an ATP-generating system (creatine phosphate (Cr ~ P)/creatine phosphokinase) decreased the activity during short-term experiments. This inhibitory effect was not found when the same ATP-generating system was added to bacteroid suspensions derived from leguminous nodules We report here the seemingly aberrant behaviour of the added ATP-generating system, the applicability of our method to other actinomycete-woody plant symbioses, and the separation of actively acetylene-reducing endophyte material from nodule homogenates.

To get a better insight into the effect of an added ATP-generating system on the nitrogenase activity of endophyte suspensions of *A. glutinosa*, the reaction was followed over an extended period. As an ATP-generating system we used phospho(enol)pyruvate (PEP) and pyruvate kinase (PK) Figure 1 shows that between 30 min and 8 h acetylene was reduced at equal rates when either ADP or ATP was added to nodule homogenates with PEP and PK. Because ADP only produced no nitrogenase activity, we concluded that the ATP-generating system can function in nodule homogenates of *A. glutinosa*. Within the first 2 h, however, the rate of acetylene reduction in complete systems was lower than that in the control. These results show that at least during the first 2 h of incubation there was no apparent inhibition of nitrogenase by ADP produced from ATP. This is in contrast to results obtained with bacteroid suspensions derived from leguminous nodules[4,5].

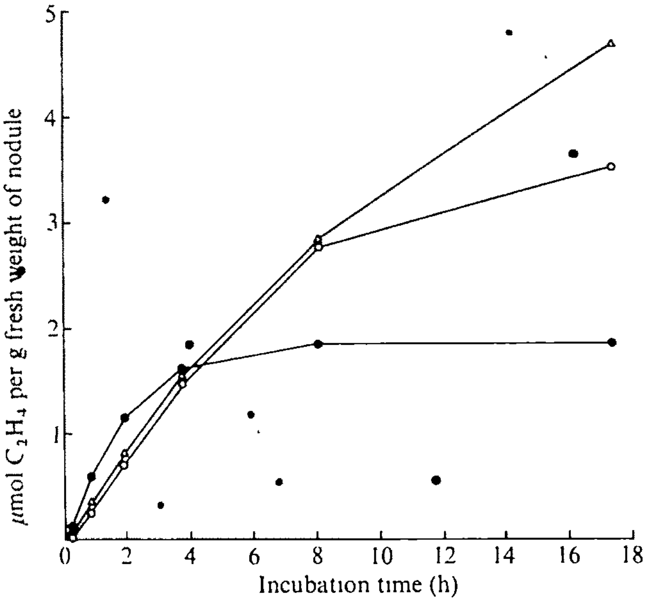

Fig. 1 Effect of PEP (10 mM) and PK (0.01 mg ml^{-1}) on acetylene reduction by nodule homogenate of *Alnus glutinosa*. General procedure for homogenisation, filtration and incubation as described previously[3], with the following modifications: 2 min homogenisation at 31,000 r p m in the presence of 100 mM dithionite. The 100-μm filtrate is referred to as the nodule homogenate. The assay mixture (2 ml) contained 1 ml of filtrate (corresponding to 14 mg of fresh nodule tissue), 2% PVP, 0.15 M sucrose, 100 mM $Na_2S_2O_4$, 2.5 mM 1,4-dithioertythritol, 50 mM Tris–HCl (*p*H 8.0), and 5 mM $MgCl_2$ Where added, ATP and ADP were 5 mM △, ATP, PEP and PK, ○, ADP, PEP and PK, ●, ATP.

It was not clear why addition of the ATP-generating system initially inhibited nitrogenase activity (Fig. 1), and so the effect of the high-energy phosphate donor was tested. Table 1 shows that

Table 1 Effect of creatine phosphate (Cr ~ P) and phospho(enol) pyruvate (PEP) on acetylene reduction by nodule homogenates of *Alnus glutinosa*

PEP (mM)	Cr ~ P (mM)	Acetylene reduction (μmol C_2H_4 per h)‖
0	0	1.20*
0	1	1.11*,†
0	5	1.00†,‡
0	10	0.82‡,§
1	0	0.93‡
5	0	0.73§
10	0	0.65§

Procedures for homogenisation and incubation are described in Fig 1. All incubation mixtures contained 5 mM ATP Means followed by the same reference mark are not significantly different by Tukey's ω procedure at 1% level

‖Calculated per g fresh weight of nodule.

Table 2 Acetylene reduction by excised nodules and by nodule homogenates of *Alnus*, *Hippophae*, *Shepherdia* and *Myrica* spp

	$Na_2S_2O_4$ during incubation (mM)	Incubation period (min)	Acetylene reduction (μmol C_2H_4 per h)§ *A glutinosa*	*H rhamnoides*	*S canadensis*	*M gale*
Intact	—	0–6	20.41	11.22	—	0.24
nodules*	—	6–12	18.59	13.40	2.87‡	1.39
Nodule†	25	0–20	4.04	2.51	0.97	0.40
homogenate	50	0–20	4.86	3.23	1.18	0.50
	100	0–20	5.19	3.13	1.13	0.60
	150	0–20	4.66	2.70	0.92	0.72

*The rate of acetylene reduction by excised nodules was measured after 6 and 12 min of incubation in 10% C_2H_2 in air at 22 °C *Alnus* and *Hippophae* spp were grown in the greenhouse, *Shepherdia* and *Myrica* spp. came from the field.

†Methods as described in Fig. 1, with the exception that the concentrations of both ATP and Mg^{2+} were 15 mM The reaction was stopped after 20 min by adding 0.5 ml 6 N HCl

‡Incubation period 3–12 min.

§Calculated per g fresh weight of nodule

both $Cr\sim P$ and PEP inhibited acetylene reduction. The concentrations used were rather low compared with those given in the literature concerning ATP-generating systems for measuring *in vitro* activity of nitrogenase (38–50 mM $Cr\sim P$ (refs 4, 5)) Higher concentrations inhibited the activity even more. The nature of this inhibition is not yet known.

Since addition of an ATP-generating system had no positive effect in short-term experiments we tried to enhance acetylene reduction with increasing ATP and Mg^{2+} concentrations (Fig. 2). Maximum levels of activity were achieved at 10 mM ATP ($ATP/Mg^{2+} = 1$). There was no significant difference (0.01 level of significance) in activity when the concentrations were increased two or fivefold. The values for nitrogenase activity shown in Fig. 1 are considerably higher than those reported earlier[3], when suboptimal levels of ATP and Mg^{2+} were used.

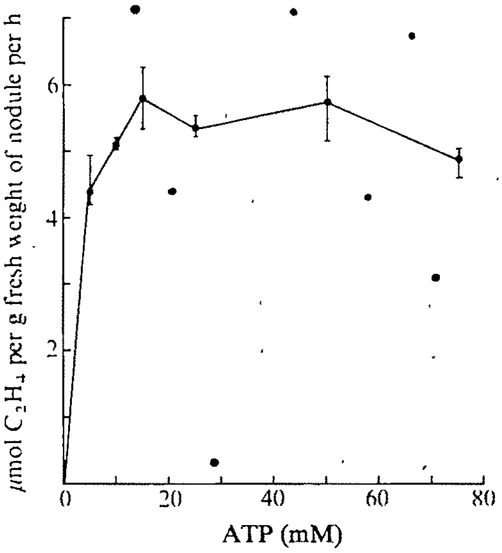

Fig. 2 Effect of ATP concentration on acetylene reduction by nodule homogenate of *Alnus glutinosa* $ATP/Mg^{2+} = 1$ Vertical brackets denote the extreme range of four replicates.

Using a constant ATP concentration of 15 mM we tested the applicability of our homogenisation and incubation method to a series of actinomycete–plant symbioses Because of the unexpectedly high optimum concentration of dithionite for *A. glutinosa*, we tried a range of concentrations. Table 2 shows that significant nitrogenase activities of nodule homogenates of various species can be demonstrated in this way and the optimum dithionite concentration seems to be different for these four types of nodules The differences in recovery (±25% for *Alnus* and *Hippophae* spp, and ±40% for the other two species) result from the differences in activity of the detached nodules This conclusion is based on observations with alders.

To study the metabolic processes in the endophyte, it is necessary to separate the intact endophyte from the host The most prominent structures of the endophyte are the vesicle clusters As these clusters show a strong tetrazolium-reducing activity it has been suggested[6] that they might also be involved in nitrogen fixation. We have developed a method to collect and purify these structures by anaerobic filtration of nodule homogenates[3,6].

In preliminary experiments, however, acetylene reduction by the fraction containing the vesicle clusters was less than 5% of the activity of the nodule homogenate[3] We have now found that the homogenisation procedure used previously was too rough and too many vesicle clusters were disintegrated. Using a milder procedure (Virtis 45 homogeniser, 4 min at 3,000 r.p.m) more vesicle clusters remained intact. Fractionation was improved further by collecting the vesicle clusters on a 10-μm filter By homogenising nodules of *A glutinosa* in this way, an activity of 2.59 μmol of C_2H_4 (calculated per g fresh weight of nodule per h, being 60% of the activity of the nodule homogenate) was obtained by the resuspended residue from the 10-μm filter. The 10-μm filtrate, consisting of hyphal fragments, disrupted vesicle clusters and plant cell debris, contained 29% of the activity of the homogenate, which was all particle bound. The remaining 11% of the activity was lost during the procedure. Results were similar with *Hippophae rhamnoides*, although the activity of the resuspended residue from the 10-μm filter was only 35% of the activity of the nodule homogenate. We think that the high recovery of nitrogenase activity in the fraction containing the vesicle clusters is an additional indication that nitrogenase is indeed located in the vesicle clusters.

As it is now possible to collect metabolically active vesicle clusters on a large scale, attention will be focused on these structures. Their relatively high nitrogenase activity *in vitro* opens the way for further physiological and biochemical studies

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Adenylate cyclase activity oscillations as signals for cell aggregation in *Dictyostelium discoideum*

As well as acting as a chemotactic factor[1] cyclic AMP stimulates cells of *Dictyostelium discoideum* to differentiate after the end of growth into aggregation-competent cells. These differentiation signals have a temporal pattern: pulses are efficient signals, whereas continuous administration of cyclic AMP may even have an adverse effect[2-4]. *D. discoideum* cells can release cyclic AMP spontaneously as reiterated pulses[4], and also release cyclic AMP in response to extraneous cyclic AMP pulses[5-7], which is important for the transmission of signals in aggregation territories in form of propagated waves[8-12] The pulsatile release of cyclic AMP into the extracellular space is preceded by a sharp (about 10-fold) increase of its intracellular concentration within 1 min (ref 4) This indicates that the oscillating variable is not only transport but also net synthesis of cyclic AMP The periodic rise of the intracellular cyclic AMP concentration could be due to periodic activation of adenylate cyclase, to inhibition of phosphodiesterase, or to the oscillatory control of both enzymes. We present here evidence for sustained oscillations of the adenylate cyclase activity in signalling cells. No concomitant changes of the ATP concentration, the substrate of adenylate cyclase, were found.

Periodic activities can be recorded in *D. discoideum* by measuring the light scattering of cell suspensions[13]. These measurements provide reference points for correlating the adenylate cyclase activities in different phases of a cycle with changes in cyclic AMP concentration in living cells.

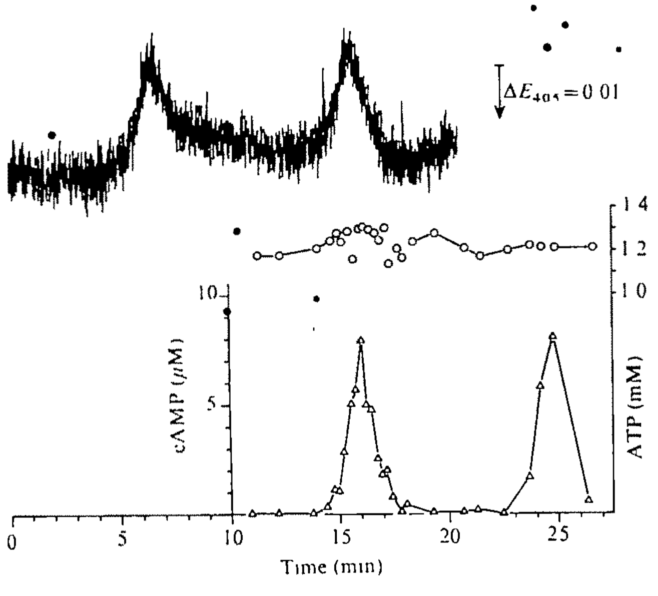

Fig. 1 Cyclic AMP (△) and ATP (○) concentrations in periodically signalling cells Changes of light scattering (top) indicate periodic activities in the cell suspension At intervals of 9 min the total cyclic AMP increases up to a sharp peak, whereas ATP in the same cells does not show significant oscillations. Because in the extracellular space cyclic AMP is rapidly degraded by phosphodiesterase[22], the cyclic AMP is predominantly intracellular and has been calculated like ATP per volume densely packed cells (left and right ordinates) Cyclic AMP was assayed by Gilman's[23] method after the addition of 5% TCA to 50 μl-samples of the cell suspension, and handling of the material as described by Gerisch and Wick[4]. ATP was extracted from suspended cells with 3% perchloric acid and determined by the bioluminiscence assay[24]. Cells of the axenic strain Ax-2, clone 206, were used 5 h after their transfer from growth medium into non-nutrient buffer. The cell density was 5 × 10[7] per ml, other conditions and light scattering measurements were as described before[13].

Spikes of decreased light scattering coincide with peak concentrations of cyclic AMP[4]. ATP concentration, measured simultaneously, did not oscillate detectably (Fig. 1): the steady-state ATP concentration was 150-fold higher than the cyclic AMP peaks. The depletion of the ATP pool of the cells due to cyclic AMP synthesis is therefore negligible, and should be rapidly compensated by respiratory control. The possibility remains that ATP, being compartmentalised, oscillates with larger amplitudes at the location of adenylate cyclase than it does on average in the whole cell.

The cyclic AMP concentration rises for not longer than 2 min in each cycle (Fig. 1), indicating similarly short phases of adenylate cyclase activation. Using the method of Salomon *et al.*[14], we reduced the assay time to 15 s, facilitating the detection of periodic short-term increases of the adenylate cyclase activity (Fig. 2). During the enzyme assay any regulatory influences due to varying concentrations of either the substrate or the product in different phases of a cycle were eliminated by adding both ATP and cyclic AMP in sufficiently high concentrations to cell homogenates.

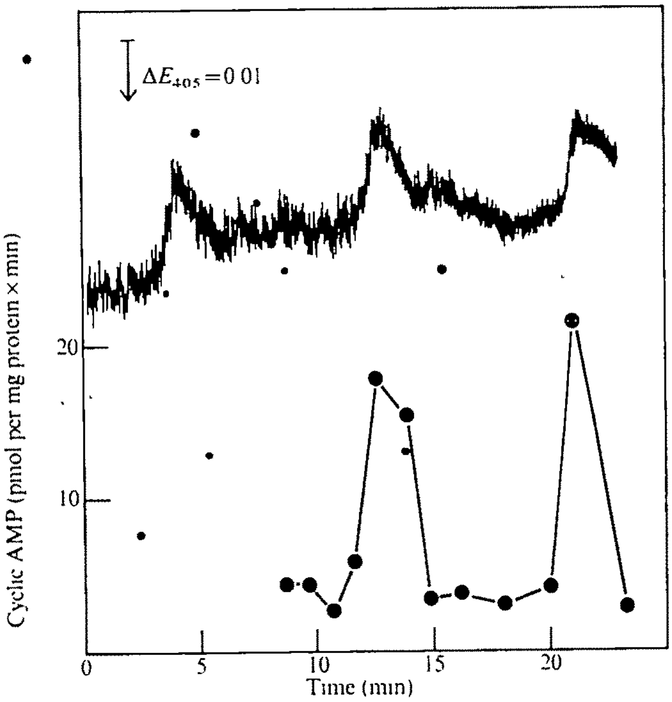

Fig. 2 Oscillations of adenylate cyclase activity in a cell suspension. Samples for the adenylate cyclase assay (bottom) were taken during continuous recording of light scattering (top). The cells were cultivated and prepared as described before[13] and used 4 h after transfer from growth medium into non-nutrient buffer. The strain was Ax-2 clone 206, and the cell density 1 × 10[8] ml^{-1}. The adenylate cyclase assay was performed according to Salomon *et al.*[14] after sonication of the cells for 5 s as described by Roos and Gerisch[16] The ATP concentration was 0.18 mM, the cyclic AMP 1 mM, and homogenate protein 1 mg ml^{-1}.

High enzyme activities in homogenates, and cyclic AMP peaks in intact cells were seen during the same part of a cycle, as indicated by the coincidence with the spikes of decreased light scattering in cell suspensions (Figs 1 and 2). In each period the enzyme stayed activated for 2–3 min, as expected from the length of time during which the cyclic AMP concentration rises in intact cells. The adenylate cyclase then returned to basal activities (Fig. 2), in accord with the sharp fall of the cyclic AMP concentration in living cells (Fig 1). The latter certainly results from deactivation of adenylate cyclase, from cyclic AMP transport into the extracellular medium[4], and perhaps to a minor extent from the action of intracellular phosphodiesterase.

In homogenates the activated state of the adenylate cyclase decayed within 1 min (Fig. 4). It remains to be seen whether the mechanism of deactivation is the same in the homogenate as in intact cells It is obvious, however, that degradation of adenylate cyclase is not the reason for the decay of its peak activities in homogenates, since the basal activity remained almost constant for a longer period (Fig. 4).

From measurements on 6 cycles a mean basal activity of 4.2±0.8 pmol cyclic AMP per mg protein per min was obtained, and a mean activation during pulse formation by the factor 7 1±1 6 (Fig. 3) In the best cases the amplification factor was 10, and the peak activities were 40–50 pmol per mg protein per min. These adenylate cyclase activities obtained in cell homogenates are comparable with the rates of cyclic AMP increase measured in living cells. A maximal rate of about 70 pmol per mg protein per min can be calculated from Fig. 1. Previously reported values have been not higher than 300 pmol per mg protein per ml (ref. 15).

What are the mechanisms of adenylate cyclase regulation? In cells which have not yet started to oscillate, adenylate cyclase is transiently activated by pulses of extraneous cyclic AMP which acts on cell surface receptors[16]. In spontaneously oscillating cells a postive feedback loop may connect cyclic AMP release, the activation of cell surface receptors, and the activation of adenylate cyclase. In fact, precocious pulses and phase shifts induced by small pulses of extraneous cyclic AMP, demonstrate a connection between the receptors and the system that periodically activates adenylate cyclase[13] This focuses attention on the regulatory pathway from the cell surface receptors to adenylate cyclase. One possibility is that upon receptor activation a soluble intracellular effector is formed, alternatively the enzyme might undergo covalent modification, for example by phosphorylation. Calcium, which at high concentrations inhibits adenylate cyclase[17] is a possible soluble effector. It seems unlikely, however, that periodic calcium sequestration is the mechanism which causes the enzyme activity to oscillate. Calcium 8 5 mM inhibited the adenylate cyclase in both its activated and non-activated state EGTA 3 mM did not prevent the decay of the activated state in cell homogenates Furthermore, when added to a homogenate with basal adenylate cyclase activity, EGTA did not activate the enzyme An earlier report[18] on the activation of adenylate cyclase by 5′-AMP has not been confirmed by recent work[16,17]

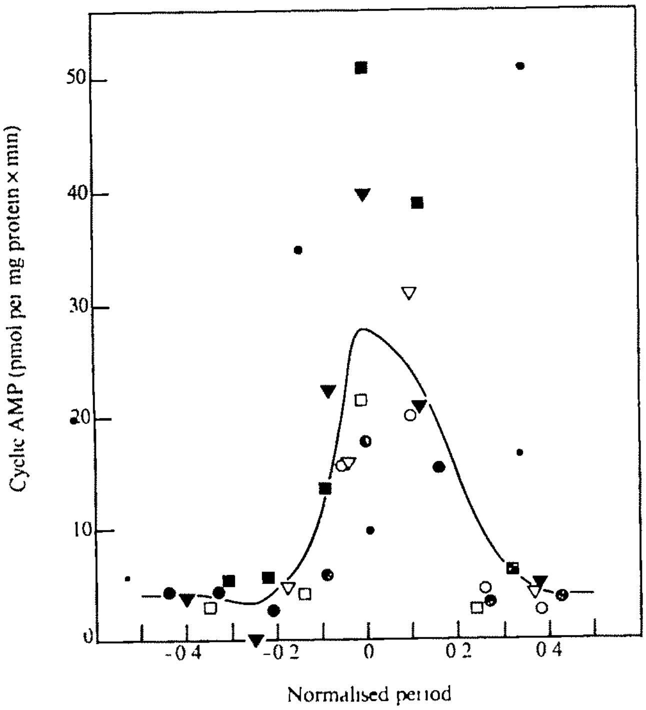

Fig. 3 Time course and amplitude of adenylate cyclase activation as the average (smooth curve) from 6 spontaneous pulses (symbols). The mean period was 7.8±0.7 min. The periods were normalised and adjusted to the peaks of decreased light scattering (zero time). Methods were as in Fig. 2 Cells of clones 206 or 214 of strain Ax-2 were used at 3–4 h after replacement of the growth medium by non-nutrient buffer.

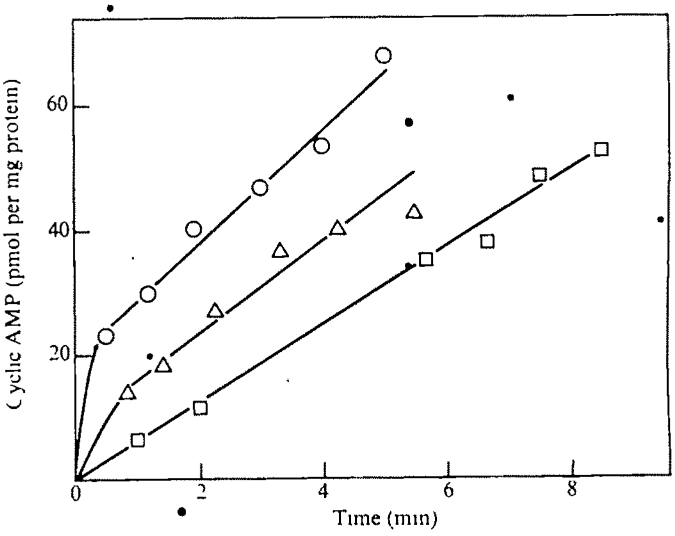

Fig. 4 Cyclic AMP synthesis in homogenates prepared at a peak of adenylate cyclase activity (○), at the phase 3 min before (□) and in a 1 : 1 mixture of both (△) The initial rate of synthesis in the mixture was the average of the basal activity found in the non-activated state, and of the high initial rate characteristic of the activated state. The activity remaining after the decay of the activated state was similar to the basal activity. Methods were as for Fig. 2, except that the concentration of ATP was 0.5 mM, and of protein 1.8 mg ml^{-1}.

To test for any soluble effector, homogenates containing activated and non-activated adenylate cyclase were mixed. The enzyme activity in such a mixture was the average of the activities in the individual homogenates (Fig. 4). This renders the presence of a soluble activator or inhibitor in homogenates from either one phase of the cycle unlikely, and favours the possibility of reversible covalent modification of adenylate cyclase.

In summary, the periodic activation of an enzyme, adenylate cyclase, is a key event in the generation of intercellular signals in *D discoideum*. The finding that the total cellular ATP does not oscillate except within the limits of experimental error, poses certain restrictions on an allosteric model of adenylate cyclase oscillations in which substrate oscillations are essential[19] The *D. discoideum* oscillator differs from chemical and biochemical oscillating systems, like the Belousow–Zhabotinsky reaction[20] or glycolysis in yeast extracts[21], in that it is composed of two compartments separated and connected by the cell membrane. In these compartments cyclic AMP oscillates with different amplitude and phase[4] Cyclic AMP is produced intracellularly, is then released into the extracellular space, from where it feeds back to its own synthesis via cell surface receptors. This opens the possibility that the cell membrane constitutes an integral part of the oscillator, functioning both in cyclic AMP transport from the inside to the outside, and as a signal transducing element in the opposite direction.

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Bacterial tumble regulator may be inactivated by methylation

KORT *et al.*[1] have described the methylation of a membrane protein which seems to be involved in bacterial chemotaxis: they show that addition of attractants affects the methylation, that the source of the methyl group is methionine, a compound required for chemotaxis, and that certain non-chemotactic mutants fail to carry out the methylation reaction. There is no relationship, however, between the amount of methylation and the tumble frequency, and the exact role of this methylation, therefore, remains mysterious Control of the tumbling process is essential for chemotaxis; peritrichous bacteria swim in short straight-line runs that are separated by abrupt changes of direction (tumbles). In the presence of attractants, however, the tumbling frequency either decreases or increases as the bacterium swims up or down the concentration gradient, respectively[2] Runs are due to counter-clockwise rotation of the flagella, whereas clockwise rotation causes tumbling[3] Aswad and Koshland[4] have proposed the existence of a "tumble-regulating substance" that would control the direction of the rotation· clockwise rotation of the flagella (and therefore tumbling of the bacteria) occurring when the concentration of this regulating substance falls below a certain threshold. The same authors have suggested that *S*-adenosyl-methionine, the product of the reaction of one molecule of methionine with one molecule of ATP[5], might be necessary for the degradation of the tumble regulator I propose that the methylated protein described by Kort *et al.*[1] may be the inactivated tumble regulator.

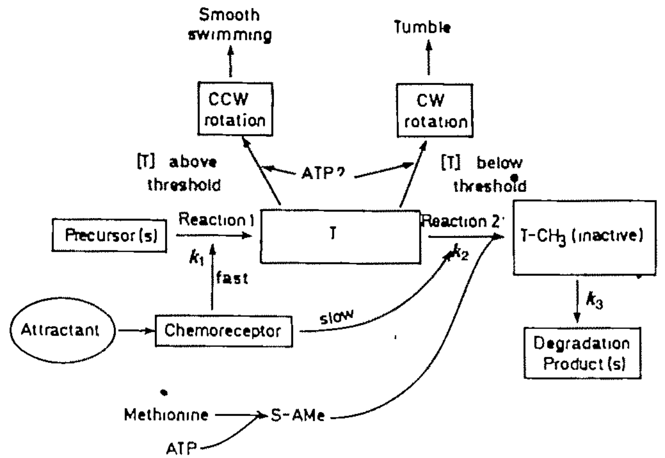

Fig. 1 Proposed scheme for inactivating tumble regulator T, tumble-regulating protein, T-CH$_3$, methylated protein, S-AMe, *S*-adenosyl-methionine; CCW, counterclockwise, CW, clockwise ATP?, ATP may be necessary for the direct control of the direction of flagella rotation[2]

Table 1 Effect of mutational changes of k_1, k_2 and k_3 on swimming behaviour and methylation

Mutation		[T]	Mobility	[T-CH$_3$]
k_2 ↑		↑	Smooth	↑
k_2 ↓		↓	Tumbling	↓
k_1 ↑		↓	Tumbling	Normal
k_1 ↓		↑	Smooth	Normal
k_3 ↑		—	Normal	↓
k_3 ↓		—	Normal	↑
k_1 and ↑ k_2	k_1/k_2 unch	—	Normal	↑
	k_1/k_2 ↑	↑	Smooth	↑
	k_1/k_2 ↓	↓	Tumbling	↑
k_1 and ↓ k_2	k_1/k_2 unch.	—	Normal	↓
	k_1/k_2 ↑	↑	Smooth	↓
	k_1/k_2 ↓	↓	Tumbling	↓
k_2 ↓, k_3 ↓		↓	Tumbling	↑
k_2 ↓, k_3 ↑		↑	Smooth	↓

T, tumble regulator, T-CH$_3$, methylated tumble regulator, Pr, precursor(s), S-AMe, *S*-adenosyl-methionine, unch, unchanged At the steady state

$$[T] = k_1[Pr]/k_2[S\text{-}AMe] \quad [T\text{-}CH_3] = k_1[Pr]/k_3.$$

Mutations implying other combinations of constant variations can be visualised as well as mutations influencing the concentrations of S-AMe and of the precursor(s)

In my proposed scheme, I have made use of the mechanism described by Macnab and Koshland[6] and by Springer *et al.*[7]—that combination of an attractant with the chemoreceptor increases k_1 rapidly and k_2 slowly This causes a transient increase in the concentration of the tumble regulator and, consequently, a transient repression of the clockwise rotation of the flagella (and thus of the tumbles).

My general scheme offers an explanation for the following experimental facts The effect of methionine starvation on methionine auxotrophs[1,4,7,8] or of the addition of arsenate to wild strains[9] is that no *S*-adenosyl-methionine is formed: reaction 2 does not take place, protein T accumulates and the bacteria do not tumble Accordingly, no methylated protein is formed and no chemotaxis can take place, which is in agreement with experimental data.

Variation of the constants k_1, k_2 and k_3 may explain the occurrence of the various types of mutants described by Kort *et al.*[1] (see Table 1) Tumbling mutants with increased methylation and smooth-swimming mutants with decreased methylation are the most difficult to account for, as one must assume the concomitant variation of two of the three constants involved.

As a consequence of the increase in both k_1 and k_2, induced by the addition of an attractant[6,7], the concentration of the methylated protein also increases, as observed by Kort *et al.*[1]. The observation, however, that, within 10 min, the concentration of the methylated protein returns to its steady-state level, remains unexplained.

If my proposed idea is true, that methylation of the tumble regulator by *S*-adenosyl-methionine causes its inactivation, then it seems that the same principle has been utilised much later in evolution, since it is known that adrenaline and noradrenaline are inactivated by catechol-*O*-methyl transferase, an enzyme which utilises *S*-adenosyl-methionine as a methyl donor[10,11].

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Mucosal penetrability enhanced by serum-derived antibodies

ALTERED mucosal penetrability for food constituents and microbial components probably underlies the pathogenesis of a variety of diseases occurring in mucous membranes and distant sites such as liver and joints[1]. The secretory immune system seems to be of major importance in mucosal exclusion of foreign material and is stimulated by topical antigens[2]. Conversely, parenteral immunisation almost exclusively gives rise to circulating antibodies which are chiefly of the IgG class. IgG is not a secretory immunoglobulin in man, monkey, rabbit and guinea pig[3], and contradictory information has been published about the effect of parenteral immunisation on the absorption of antigen through mucous membranes. Moreover, a possible influence on the concurrent mucosal penetration of unrelated macromolecules has not been considered previously. We report here that although serum-derived antibody retards the penetration of corresponding antigen, it nevertheless causes impairment of the mucosal barrier. Induction of IgG antibodies may thus cause hazardous side effects by altering mucosal penetrability.

Mucosal specimens devoid of a secretory immune system were obtained from the lingual frenum of 16 adult Belted Dutch rabbits, 8 of which had been immunised subcutaneously over a period of 2–3 months with a total of five 3-mg doses of human albumin emulsified in complete Freund's adjuvant. This schedule resulted in high levels of precipitating serum antibody (Table 1), which by immunoelectrophoresis was shown to be located chiefly in the IgG fraction. The excised membranes were rinsed in saline and mounted in diffusion chambers[4] with the oral side exposed to 1 ml tissue culture medium containing 30 mg human albumin and 30 mg human transferrin, and with the basal side exposed to 200 μl medium alone. After incubation for 2 h in a humid atmosphere of CO_2 in air at 37 °C, the basal concentrations of albumin and transferrin were measured. In preliminary experiments this was attempted by isotope labelling which, however, was found to be unreliable[5]. Since radioimmunoassay has also been found unreliable in similar experiments[6], the two components were quantified by single radial immunodiffusion performed essentially as described before[7]. To obtain sufficient sensitivity (detection limit of about 0.25 μg ml^{-1}), specific rabbit antisera were used at final dilutions of 1:300 (anti-albumin) and 1:160 (anti-transferrin) in the Agarose gel, and the 5-μl sample wells were refilled three times. The precipitin rings were intensified by overlaying the gel with filter paper soaked in 1:3 dilution of goat antiserum to rabbit IgG.

Table 1 shows that the control membranes discriminated between albumin and transferrin, according to the difference in molecular weights (69,000 and 90,000), as reported for other biological membranes *in vivo*[8]. This held true even for the specimen showing exceptionally high penetrability. Its defect was probably restricted to an epithelial damage, since penetration studies with rabbit intestinal mucosa have indicated that molecular selection takes place at the basement membrane whereas the epithelial layer is solely rate limiting[9].

The penetration of albumin through membranes from immunised animals was reduced whereas the concurrent penetration of transferrin was enhanced (Table 1). The former result supports previous experiments performed with intestinal and oral mucosa of parenterally immunised animals[10,11], and may be ascribed to immune complex formation. Binding of antigen to antibody in the mucus covering the intestinal surface has been suggested[12], whereas for the oral mucosa (gingival crevicular epithelium) ultrastructural indication of immune complex formation within the epithelial interstices has been presented[11]. Ultrastructural studies of small intestinal mucosa, however, have indicated increased antigen absorption into epithelial cells after parenteral immunisation[13].

Thus, the level at which serum-derived antibody retards mucosal antigen penetration has not been clearly defined. Since some IgG diffuses through epithelial interstices[14,15], immune complexes could be formed at the epithelial level, but also in the connective tissue. Exposure of epithelial surfaces to antigen in sensitised animals results in a rapid local emigration of neutrophils[11,16]. Phagocytosis of immune complexes and degradation of antigen could therefore contribute to the apparently retarded mucosal antigen penetration. Moreover, release of enzymes from these cells probably explains the most important finding in our experiments—the enhanced penetration of an unrelated macromolecule. It is well known that neutrophil lysosomal enzymes can

Table 1 Effect of parenteral immunisation with albumin on the concurrent penetration of albumin and transferrin through rabbit oral mucosa

	Serum antibody*		Basal concentration as % of oral concentration† Albumin		Transferrin	Significance of difference‡
Control animals			(0.0215)§		(0.0155)	
			0.0061		0.0034	
			0.0054		0.0018	
			0.0047		0.0035	
			0.0035		0.0008	
			0.0020		0.0009	
			0.0005		0.0005	
			0.0005		0.0005	
		Mean	0.0032	Mean	0.0016	$P<0.025$
Immunised animals	8		(0.0037)		(0.0131)	
	2		0.0013		0.0025	
	32		0.0010		0.0055	
	16		0.0005		0.0076	
	32		0.0005		0.0032	
	16		0.0005		0.0020	
	16		0.0005		0.0018	
	16		0.0005		0.0016	
		Mean	0.0007	Mean	0.0035	$P<0.01$
Significance of difference‖			$P<0.03$		$P<0.05$	

*Expressed as precipitating units based on tests against albumin (1 mg ml^{-1}) in gel diffusion
†Each figure is an average of 2–4 measurements (detection limit 0.0008%, undetectable assigned 0.0005%)
‡Wilcoxon matched-pairs signed-ranks one-tailed test
§Figures in parentheses excluded from group calculations because of the extreme deviations, apparently reflecting a membrane defect
‖Mann–Whitney one-tailed *U*-test

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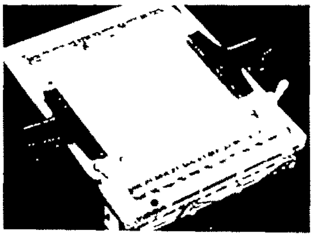

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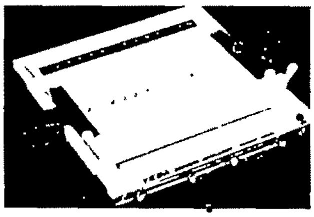

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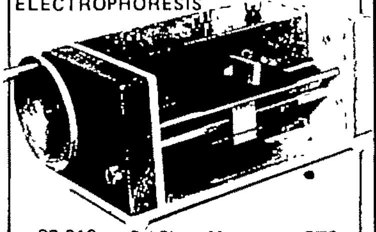

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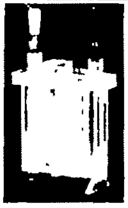

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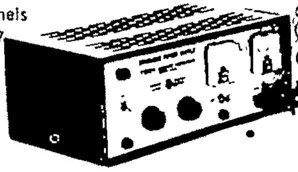

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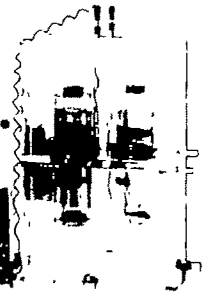

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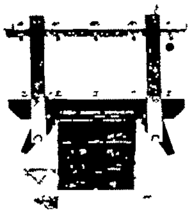

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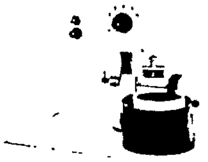

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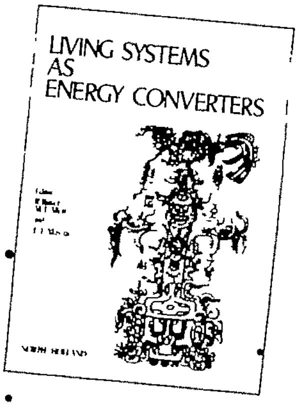

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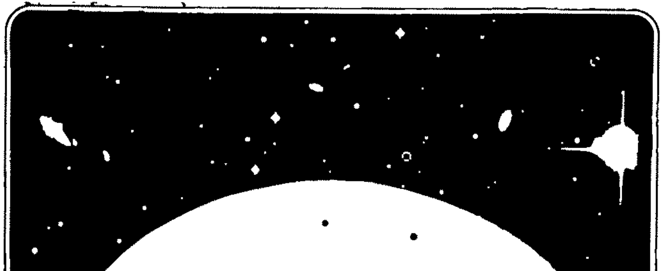

Galaxy Formation

John Gribbin

A personal view of galaxy formation – a subject which holds a central place in modern astrophysics. The book reflects the author's own interests in this field, and thus offers a new and different perspective to a fascinating topic. Formal mathematics is kept to a minimum and the general level of the text makes it suitable for the second year physics students at university.

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affect basement membrane zones and the ground substance[17], and mucosal penetrability may thereby be increased.

Several possible hazards of circulating antibodies resulting from vaccination have been discussed[18]. Our results point to one way in which IgG antibodies may jeopardise mucosal integrity since undue penetration of new antigens in turn may stimulate a broader IgG response and thus set up a vicious circle. The merit of the selective glandular secretion of IgA and IgM antibodies[15] is probably to limit such a development. This must be taken into account when using vaccines in immunoprophylaxis and when the value of breast feeding is debated. In the neonatal period antigens are absorbed into the circulation in sufficient amounts to evoke a systemic IgG response in a great proportion of individuals, especially in those being artificially nourished[19]. This may predispose to untoward sensitisation of the child In addition, our results substantiate one pathogenetic potential of local IgG-cell responses in mucosal disease[14,15].

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Endplate current fluctuations reveal only one channel type at frog neuromuscular junction

SIGNAL transduction between nerve and muscle is mediated by a chemical process at the neuromuscular junction In the nerve ending electrical activity causes the release of acetylcholine (ACh) into the synaptic cleft separating the nerve and muscle cells. ACh can bind to specific chemical receptors in the postjunctional muscle membrane; these receptors control a gating mechanism that can increase membrane conductance locally Normal nervous activity triggers an action potential and contraction in twitch muscle fibres. At constant membrane potential the membrane conductance change induces a current that is carried largely by Na^+ and K^+ in frog[1], moving through ACh-gated membrane channels. There has been a controversy regarding the nature of the chemically gated endplate channels, are there kinetically-independent channels for Na^+ and K^+, or are these ions transported by the same channel? Maeno[2] initially proposed separate endplate channels, arguing that the two component decay of endplate potentials in procaine-treated cells resulted from a selective effect on Na^+ channel kinetics Kordas[3,4] and Magleby and Stevens[5] argued that Maeno's two-channel hypothesis could not account for the unique reversal potential observed or the monotonic voltage dependence of endplate current (EPC) decay. The analysis below of iontophoretically-induced EPC fluctuations in procaine-treated and untreated preparations indicates that the ions which constitute the EPC are not carried by kinetically distinguishable endplate channels.

Our conclusions are based on the following quantitative description of the EPC. Consider a population of identical, independently functioning endplate channels in voltage clamp conditions at membrane potential V. When ACh is iontophoretically applied an EPC, μ_I, will flow in the open channels

$$\mu_I = \gamma P N (V - V_r) \tag{1}$$

where γ is the mean conductance of one open channel, P is the probability that any single channel will open when the endplate is exposed to ACh, N is the total number of channels, and V_r is the reversal potential at which there is no net current. The EPC will fluctuate if the application is prolonged, because individual channel lifetimes are of the order of several milliseconds[6] The fluctuations are caused by changes in the actual number of conducting channels reflecting the probabilistic nature of channel opening and closure[7] The intensity of the fluctuations may be characterised by their variance, σ^2, about the mean EPC

$$\sigma_I^2 = \gamma^2 P(1-P) N (V - V_r)^2 \tag{2}$$

Note that P is a function of ACh concentration which is non-uniform over the extended frog endplate. A more complex derivation which takes this spatial dependence into account produces essentially these same relations

If the EPC were carried in separate Na^+ and K^+ channels, similar treatment would give the mean and variance of the EPC as

$$\mu_{II} = \gamma_{Na} P_{Na} N_{Na} (V - V_{Na}) + \gamma_K P_K N_K (V - V_K) \tag{3}$$

$$\begin{aligned}\sigma_{II}^2 = {} & \gamma^2_{Na} P_{Na} (1 - P_{Na}) N_{Na} (V - V_{Na})^2 + \\ & + \gamma^2_K P_K (1 - P_K) N_K (V - V_K)^2\end{aligned} \tag{4}$$

Here the subscripted variables have meanings similar to those described above. The approximate equilibrium potentials of Na^+ and K^+ in frog muscle are $V_{Na} \simeq +40$ mV, $V_K \simeq -100$ mV. Their exact values are unimportant as long as it is noted that they clearly differ from the observed reversal potential, $V_r \simeq 0$ mV.

The two schemes may be distinguished experimentally by measuring the EPC variance at the observed reversal potential. For the single channel scheme at $V = V_r$, the net EPC is zero because the net current in each channel is zero (equation (1)); likewise the variance is zero (equation (2)). For the two-channel scheme at $V = V_r$, the net EPC is zero because the inward Na^+ current is equal but opposite to the outward K^+ current (equation (3)), however, the variance is non-zero (equation (4)) since the Na^+ and K^+ terms are each positive and cannot cancel.

EPCs were generated in voltage-clamped muscle cells of m cutaneous pectoris from the frog *Rana pipiens*. Nomarski and modulation contrast optics were used to observe the endplates, and ACh was applied by iontophoresis 10–20 μm from the cell. Normal Ringer's solution contained 100 nM tetrodotoxin whereas procaine–Ringer's did not; composition (mM). NaCl, 117.2, KCl, 2.5; $CaCl_2$, 1 8; Na_2HPO_4, 2.16; NaH_2PO_4, 0.85 (*p*H 7.0). The induced EPCs were processed by a digital laboratory computer The variance was estimated as the frequency integral of the mean spectral density of the digitised current fluctuations, taking care to exclude miniature EPCs. The methods were similar to those of Dionne and Stevens[8] and Ruff[9].

Figure 1 contains data from a cell which was not treated with local anaesthetic The mean EPC and its variance are plotted as a function of membrane potential, and it is seen that the variance approaches zero at the measured V_r. Similar observations were made on 22 untreated cells and 7 cells in 3×10^{-4} M procaine from 13 different frogs. In all cases the variance at the reversal potential was less than the uncertainty in its measurement and was typically about 2 orders of magnitude below its value at −60 mV

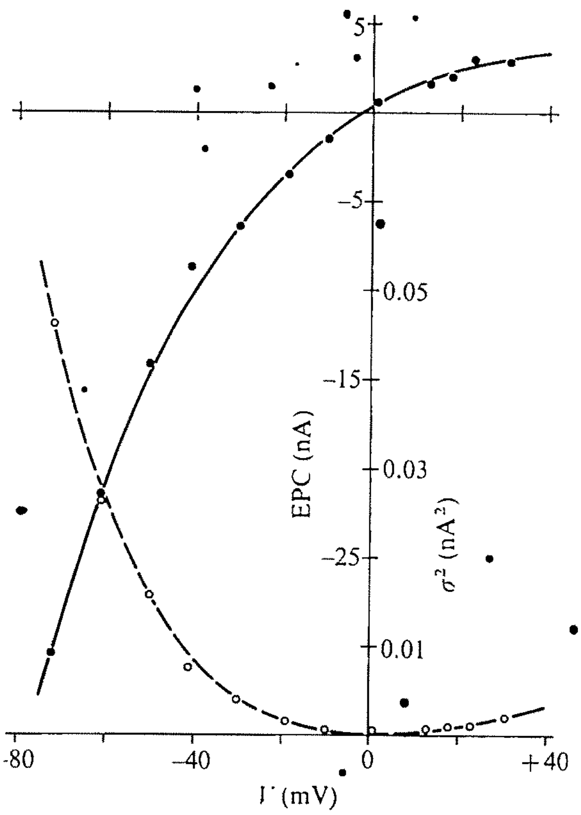

Fig. 1. Mean EPC and variance of EPC fluctuations about their mean are shown as a function of membrane potential, V. The smooth curve is equation (5) fitted to the EPC data to evaluate A, B and V_r. The values used here for the best fit are $A = -15.36\ V^{-1}$, $B = 144$ nA and $V_r = -2$ mV The same values are used to fit the voltage dependence of σ^2 with equation (6), the dashed curve The single-channel conductance at −70 mV was 22 pS for this fibre The quality of fit between theory and experiment in the variance measurements may be to some degree fortuitous Most cells exhibited slightly greater measured variance at positive membrane potentials than predicted; however, to establish a +30 mV membrane potential, holding currents of several hundred nanoamperes were commonly required Fluctuations in the holding current make accurate measurement of the few nanoampere EPC variations difficult. The data shown in this figure are from the cell with the best signal-to-noise ratio

These data are inconsistent with the model in which Na^+ and K^+ pass through separately gated endplate channels. The two-channel model predicts that the variance of the EPC is non-zero at the reversal potential. Ruff[9] also found that the variance of the EPC vanished at V_r for preparations exposed to the trimethyl lidocaine derivative QX-222, a different local anaesthetic.

The conclusion that a population of identical channels carries the EPC has been obtained by comparing measurement with theory at a single voltage, V_r. Equations (1) and (2) also provide descriptions of the functional dependence of EPC and its variance on voltage. Fitting these relations to the data, however, requires a more specific model of channel kinetics, because the probability of channel opening, P, is a function of voltage[8].

As has been shown by others[5,10,11], normal channel operations may be represented by the following kinetic scheme.

$$nT + R \overset{K}{\rightleftarrows} T_nR \underset{\alpha(V)}{\overset{\beta(V)}{\rightleftarrows}} T_nR^*$$

$$\alpha(V) = B_1 e^{A_1 V}$$

$$\beta(V) = B_2 e^{A_2 V}$$

where T represents agonist molecules, n of which bind to receptors R to produce at equilibrium an intermediate T_nR and an open channel T_nR^*. The probability of channel opening is voltage dependent, for the case here of prolonged agonist exposure, the dependence is exponential and given by[8]

$$P = \beta C^n/\alpha K$$

$$= [C^n B_2/KB_1] \exp(A_1 - A_2)V$$

where C is ACh concentration

Thus, equations (1) and (2) may be rewritten to express their voltage dependence

$$\mu_1 = B(V - V_r)e^{AV} \qquad (5)$$

$$\sigma_1^2 = \gamma B(V - V_r)^2 e^{AV} \qquad (6)$$

In writing equation (6) it has been assumed that $P \ll 1$, an approximation which is accurate in the low agonist concentration limit that applies here The voltage dependences of equations (5) and (6) are unchanged when a more detailed derivation includes the spatial non-uniformity of ACh concentration. Although equation (1) suggests that the EPC is proportional to voltage, we see in equation (5) a more complex relation as pointed out by Dionne and Stevens[8]. In addition, the variance (equation 6) is similarly affected by the voltage dependence of the probability of channel opening so that what seems to be a parabolic dependence in equation (2) is not so simple.

In Fig. 1 we have fitted equation (5) to the EPC–voltage data to evaluate A, B and V_r. These values were used in equation (6) which was drawn as the dashed curve through the variance measurements; the single-channel conductance γ was evaluated at −70 mV by taking the ratio of the ACh-induced conductance to its measured variance The skewing of σ^2 by the voltage dependence of channel gating appears as a steepening of the parabolic behaviour for $V < V_r$ and a depression at $V > V_r$, and this behaviour is well described by equation (6). The ability to predict accurately the variance from measurements of the EPC is another indication of the essential correctness of the quantitative description of receptor kinetics which we have used

Our description of endplate channel kinetics has been at two quantitative levels which contain certain basic assumptions in common. An elemental stochastic theory in which individual channels are represented as independent, two-state devices, either open or closed, leads us to conclude that Na^+ and K^+ currents at the endplate are simultaneously gated This result follows because the EPC variance at the reversal potential is zero, contrary to the predictions of models assuming separately gated ion currents. The most parsimonious implication is that both Na^+ and K^+ share the same endplate channel. It should be emphasised that our results pertain to the gating of the chemically induced conductance change and do not address the question of whether Na^+ and K^+ have equal conductance in the open channel.

Extending this simple theory by incorporating a specific kinetic scheme for receptor function has allowed us to predict the voltage dependence of the EPC variance with coefficients determined from the mean EPC The measured variance fits the predicted curve well over the range tested which supports the accuracy of the equations derived from the model. The kinetic model we used was selected because in the past it has accounted well for the general quantitative behaviour of the ACh-gated channel. Note that any kinetic scheme which yields quantitative predictions similar to ours would also be acceptable Our conclusion that individual EPC ionic species are not separately gated is independent of the particular kinetics.

It may be useful to note the quantitative behaviour of our description over a voltage range more extensive than we have been able to test. If V could have been raised above +100 mV both the mean EPC and its variance are predicted by equations (5) and (6) to fall towards zero again. This phenomenon of EPC rectification is mediated largely by channel lifetime which approaches zero as membrane potential is increased Since the dependence of channel lifetime on membrane potential is nonlinear, at positive voltages the shortened lifetime more than compensates for the increased driving potential on the current carrying ions and little or no EPC can be induced. A similar mechanism may account for anomalous K^+ rectification in voltage sensitive channels in both cardiac and skeletal muscle.

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Selective activation of sympathetic ganglia in young spontaneously hypertensive rats

PROLONGED increase in peripheral sympathetic discharges producing vasoconstriction leads to enhanced synthesis of tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) in terminal adrenergic neurones[1,2]. This is mediated by an increased activity of the preganglionic cholinergic nerves[1]. Young spontaneously hypertensive rats (SHR) have been used as a prehypertensive model of essential hypertension to investigate whether such peripheral sympathetic neuronal activation initiates the increase of blood pressure. Study of the prehypertensive stage gives more information on the initiation of the blood pressure elevation than study of hypertensive animals. Circulating levels of noradrenaline, and DBH activity (as measures of peripheral sympathetic nerve system activity were markedly elevated in young SHR (refs 3, 4), although contradictory results were reported for adrenomedullary activity[3-7]. In young SHR, adrenal levels of DBH, TH and phenylethanolamine *N*-methyl-transferase (PNMT) activities were lowered[3], whereas other investigators[4,7] found an elevation of adrenal DBH and PNMT activities. Transection of the splanchnic nerves markedly lowered blood pressure in young[5,6] and adult SHR (refs 5, 7), and also reduced adrenal venous output of adrenaline in conscious young SHR (ref. 5). All these findings suggest enhanced activity of the adrenal medulla and preganglionic cholinergic discharges to the adrenal medulla. We report here preganglionic nerve-dependent elevation of choline acetyltransferase (ChAc) and TH activities occurring specifically in the coeliac ganglia of young SHR.

In young SHR, the activity of preganglionic cholinergic nerve input to various sympathetic ganglia, superior cervical ganglia, stellate ganglia and coeliac ganglia, and adrenal glands was examined by measuring ChAc and acetylcholinesterase (AChE) activities as a measure of presynaptic neuronal activity[8] and TH activity as an index of postsynaptic neuronal activity[9] at the age of 4–5 weeks, when the blood pressure of SHR did not differ from normotensive controls. Normotensive animals of the Kyoto Wistar rats (KWR), from which SHRs were derived, were used as controls. Systolic blood pressures as measured by a tail plethysmographic method were 105±11 mmHg (mean ± s.e.m., $n = 5$) for young KWR and 110±7 mmHg ($n=5$) for young SHR, respectively. Ganglia and adrenals were removed and homogenised in pairs in 0.2 ml of ice-cold 0.005 M Tris buffer (*p*H 7.5) containing 0.1% Triton X-100. ChAc and AChE activities[10] and TH activity[11] in the homogenates were determined radiometrically.

In young SHR, ChAc and TH activities of coeliac ganglia were signficantly elevated as compared with those in young KWR. In the superior cervical ganglia, stellate ganglia and adrenals, however, there were no significant changes (Table 1). AChE activities in all ganglia and adrenals of young SHR did not differ significantly from those of KWR. Total protein contents of a pair of coeliac ganglia in young SHR (0.44±0.03 mg, $n=5$) did not differ from those in KWR (0.41±0.02 mg, $n=5$).

Table 2 shows the effect of sectioning the pre- and postganglionic nerves to the coeliac ganglion on its elevated ChAc and TH activities, and AchE activity. Bilateral preganglionic nerve transection markedly lowered the elevated ChAc activity to a level less than the ChAc activity of KWR 5 d later, but did not change TH and AChE activities. Bilateral post-ganglionic nerve section did not change ChAc, AChE and TH activities in the coeliac ganglion. Difference in the control values for ChAc and TH in both experiments (Tables 1 and 2) may be due to sham-operated stress as well as age difference (5 or 4 weeks). Since decentralisation of sympathetic ganglia removes synaptic input to the ganglia rather than nonspecific surgical damage[12], the present finding suggests that elevated ChAc levels are due to the enhanced ACh release and formation by the activation of preganglionic neuronal input to the coeliac ganglion of young SHR. The persistent elevation of ChAc activity in the coeliac ganglion even after the post-ganglionic axotomy negated the reflex of the innervating end-organ activity. Removal of sympathetic target organs, however, is known to prevent the normal maturation of ChAc in presynaptic endings during development[13]. Selective activation of the coeliac ganglion may be explained by an enhanced reactivity of the ganglia to pre-ganglionic nerve discharges rather than the regional elevation of nerve input. After treatment with nerve growth factor, the prevertebral coeliac

Table 1 Enzyme activities in sympathetic ganglia and adrenals of young spontaneously hypertensive (SHR) and Kyoto Wistar rats (KWR) at 5 weeks of age

Organ	Choline acetyltransferase (nmol ACh per h per pair)		Acetylcholinesterase (nmol acetate per h per pair)		Tyrosine hydroxylase (nmol CO_2 per h per pair)	
	KWR	SHR	KWR	SHR	KWR	SHR
Superior cervical ganglia	3.84±0.41	3.52±0.48	23.6±1.5	22.0±0.72	0.115±0.009	0.102±0.001
Stellate ganglia	2.86±0.26	2.98±0.31	21.7±1.2	22.8±0.77	0.081±0.009	0.094±0.016
Coeliac ganglia	2.88±0.51	4.16±0.6*	21.3±0.9	19.6±0.45	0.180±0.019	0.252±0.021*
Adrenals	11.8±0.8	13.6±1.1	62.6±3.2	66.1±3.2	0.842±0.195	1.14±0.15

Values are mean ± s.e.m. for 5 animals at 5 weeks of age.
*$P < 0.05$ compared with KWR.

Table 2 Effects of pre- and post-ganglionic nerve transection of the bilateral coeliac ganglia on its elevated choline acetyltransferase activity in young SHR at 5 weeks of age

Group	Choline acetyltransferase (nmol ACh per h per pair)	Acetylcholinesterase (nmol acetate per h per pair)	Tyrosine hydroxylase (nmol CO_2 per h per pair)
SHR, sham operated	3.27±0.32	21.3±2.3	0.167±0.009
SHR, preganglionic axotomy	0.47±0 08*	20.7±1.4	0.152±0 011
SHR, postganglionic axotomy	2.66±0.33	19.8±1.2	0.175±0.019
KWR, sham operated	2.25±0 15†	19.5±0.8	0.134±0.011†

Values are mean ± s.e.m. for 5 animals at 4 weeks of age. Axotomy was performed under ether anaesthesia 5 d before killing. Differences in control values for ChAc and TH when compared with Table 1 are probably due to the effects of age difference and laparotomy stress.

*$P < 0.01$ compared with SHR controls.

†$P < 0\ 05$ compared with SHR controls.

ganglion shows a significantly more pronounced hypertrophy than the paravertebral superior cervical ganglion and stellate ganglion in young rats[14]. Our results indicate possible selective maturation of coeliac ganglia in young SHR. This is supported by findings that there is a rostro-caudal gradient in TH ontogeny in various sympathetic ganglia, for example, in maturation of the sixth lumbar ganglia at 2 months of age[15] and in superior cervical ganglia at 14 d (ref. 16). Thus it suggests that in the coeliac ganglia, TH activity would not reach adult plateau values by 4–5 weeks of life, the age of young SHR in our experiments.

The observed activation of the coeliac ganglion in young SHR may enhance the release of noradrenaline and DBH levels into peripheral circulation from the coeliac ganglion-innervated arteries in splanchnic region and others. It is clear that in young, prehypertensive SHR, the selective activation of pre- and post-synaptic neuronal activities in coeliac ganglia initiates the elevation of blood pressure.

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Colour-coding properties of sustained and transient channels in human vision

WHEN the colour of a visual stimulus is repetitively alternated between say red and green, the percept evoked depends upon the temporal frequency of repetition At very low frequencies (for example, 1–2 Hz), the repetitive change in colour is readily apparent. But at frequencies above about 10 Hz, no changes in colour are perceived, the stimulus in this case would appear a uniform yellow but would still seem to be flickering in brightness Observations of this kind suggest that the colour of a stimulus may be analysed by a set of channels different from that analysing the brightness or flicker. The flicker or brightness channels respond to higher temporal frequencies of flicker than do the colour channels This idea was confirmed by King-Smith[1] who has described how the sensitivity to coloured flashes of light changes with flash duration He further described the subjective appearance of the flash, and concluded that a stimulus was not necessarily detected by the same channels as those that detected its colour. When the flash was brief, the stimuli usually looked achromatic, except when the wavelength was below about 490 nm The intensity of the flash had to be raised above the detection threshold before the colour could be perceived At longer flash durations, the colour was apparent as soon as the flash was detectable. The channels signalling the colour of a stimulus have a longer 'integration time' than those giving an achromatic percept. This kind of experiment examines differences in the high temporal frequency behaviour of the two sets of channels. It is of interest to examine the low temporal frequency behaviour as well because recent neurophysiological investigations of the monkey's visual system make use of this behaviour in classifying neurones[2–5]. Some neurones give a sustained response to prolonged presentation of a stationary stimulus, others respond only transiently to the stimulus onset I show here that human colour channels give sustained responses while the flicker or brightness channels give transient responses.

The stimuli were disks (1° diameter) viewed against a white background (3 5° diameter) The background, viewed through a pellicle, was made by illuminating a diffusing screen with a tungsten halide bulb of unknown colour temperature. The luminance of the background was 50 cd m^{-2} which gave a retinal illumination of about 1000 td (5 mm pupil) The light source for the 1° test stimuli was the P31 phosphor of an oscilloscope. This is not ideal as the phosphor emits little red light, so that the range of wavelengths examined is limited. Four test 'colours' were used: blue (Ilford 621; nominal peak 450 nm), green (Ilford 807; 530 nm), yellow (Ilford 626; 570 nm) and orange (Ilford 607, 590 nm). The widths of these filters at half maximum transmission are 30–80 nm.

Test stimuli were flashed on and off at intervals of 4 s while the background remained steadily illuminated. The observer adjusted the intensity of the test stimuli until he considered that they were only just visible. Eight threshold settings were made for each stimulus. In the first series of experiments, the observer attempted to detect an 8-ms flash of the stimulus superimposed on a subthreshold flash lasting 1 s. The test and subthreshold flashes were spatially co-extensive and were of the same colour. It has been argued[6] that this technique examines the subliminal response of channels to the subthreshold long flash Thus, if the subliminal response is transient, the sensitivity for the brief flash will be increased soon after the onset of the conditioning flash but will return to its control value after 100–200 ms, as the transient response subsides. But, if the subliminal response is sustained, the sensitivity will not return to its control value until after the end of the conditioning flash.

Figure 1*a* illustrates the results of such an experiment for three different colours. In all three cases the sensitivity to the test flash was increased when it appeared 16 ms after the onset of the conditioning flash. For green and orange stimuli, the sensitivity returned almost to normal when the test flash appeared 500 ms after the onset of the conditioning flash The subliminal response to the long flash was transient. For the blue flash, however, the sensitivity remained high, suggesting a sustained subliminal response

In confirmation of King-Smith's finding, the brief green and orange flashes appeared almost achromatic while the blue flash did appear a deep blue. The almost achromatic percept was of a silvery glow with perhaps a tint of the appropriate colour No attempt was made to show rigorously that the percepts evoked by the green and orange stimuli were indistinguishable.

Figure 1*c* shows the results of a similar experiment, but with test flashes lasting 250 ms. For both the green and orange stimuli, the sensitivity to the test flash remained higher than normal even when it was presented 500 ms after the onset of the long flash. The subliminal responses of the channels detecting the 250-ms flashes were sustained. Again, confirming King-Smith's observations, the test flashes (being long) appeared coloured.

These results suggest that if a narrow-band stimulus appears coloured, it is detected by sustained channels. If it seems achromatic, it is detected by transient channels. It might be expected that, if two stimuli seem to be of different colours, they would be detected by channels responding to different narrow bands of wavelength, the stimuli might be detected independently. Apparently-achromatic stimuli of different wavelength might be detected by one set of broad band channels, stimuli of different wavelength would summate.

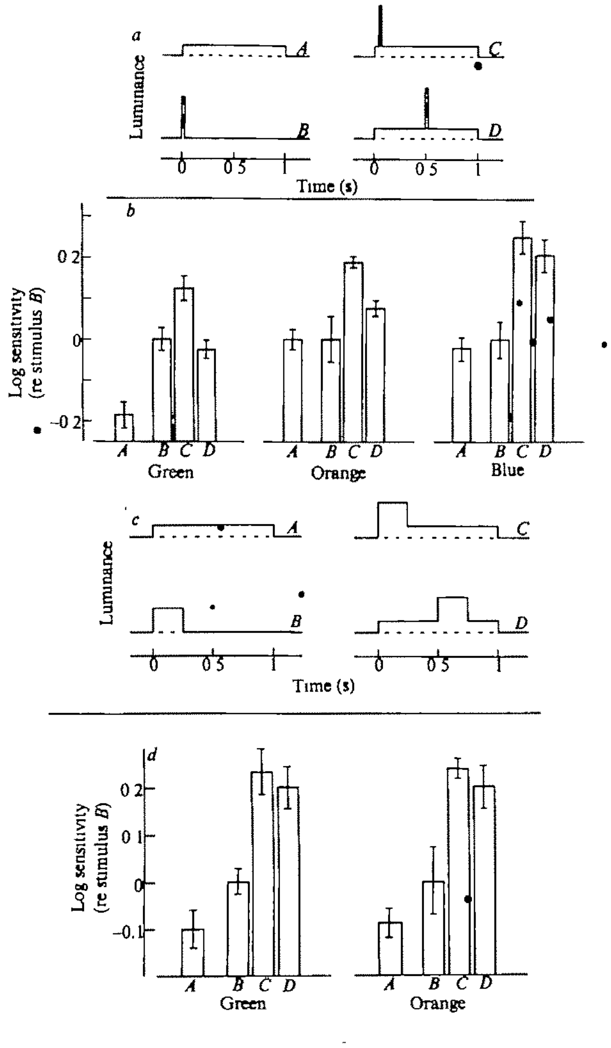

Fig. 1 Sensitivity to an 8-ms flash in the presence of a subthreshold flash lasting 1 s. *a*, Temporal waveforms of the four stimuli. Stimulus *A* is the conditioning flash alone, stimulus *B* is the test flash alone. In stimulus *C*, the test flash occurs 16 ms after the onset of the conditioning flash, in stimulus *D*, the test flash occurs with a delay of 500 ms. *b*, Sensitivities for the four temporal waveforms, for each of three colours. Sensitivity (mean) is expressed relative to that for stimulus *B* in each case. The vertical lines on each histogram block show ± 1 s.e.m. *c* and *d* are as for (*a*) and (*b*), respectively, except that the test stimuli lasted 250 ms as shown in the upper panel. Only green and orange stimuli were used.

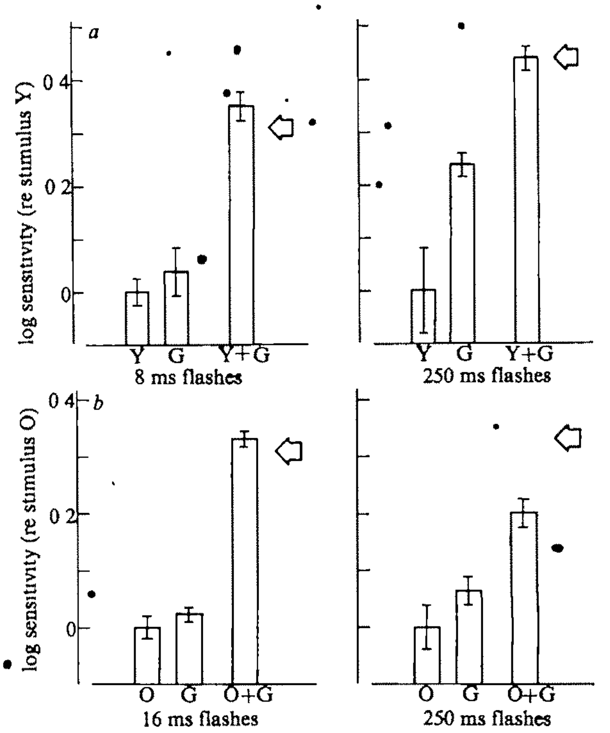

Fig. 2 Summation between stimuli with different wavelength composition *a*, for yellow (Y) and green (G) stimuli. Summation was examined at two flash durations. The yellow and green flashes in each case were synchronous and of the same duration. Sensitivity is expressed relative to that for the yellow stimulus alone. The horizontal arrows show the sensitivity for the composite stimulus if there had been perfect summation of the sensitivities to the two components *b*, for orange (O) and green (G) stimuli.

Figure 2*a* illustrates an experiment to test this prediction. Two test stimuli of different colour were generated using two oscilloscopes viewed through a half-silvered mirror. In the upper part of the figure, a yellow flash was added to a simultaneous green flash. The relative intensities of the two stimuli were adjusted to make them more or less equally detectable alone. The sensitivity to the mixture was then determined. The arrows show the sensitivity that would have been achieved if there had been perfect summation. Both for short and for long flashes, summation was more or less perfect, implying that the two colours were detected by the same channels. This was found even though the long flashes appeared coloured. The green and yellow flashes were not easily distinguishable, however, both appeared to be green at threshold, suggesting that they were detected by the same colour-signalling channels. Figure 2*b* shows a similar experiment. The wavelengths of the two test stimuli were made more dissimilar (green and orange). Perfect summation was found only for the brief flashes, when both stimuli appeared achromatic. At long durations, the flashes appeared to be of different colours, and summation was incomplete. These results seem to be consistent with the observation that summation of the brightness of different wavelengths is almost perfect in flicker photometry[7], whereas complex interactions are found when the stimuli are presented for 500 ms (ref. 8).

My results confirm King-Smith's suggestion that there are two sets of channel in the human visual system. One class signals the colour of a stimulus, this class is made up of several subsets, which each respond to a limited band of wavelengths. The second class of channel does not give information about the colour of a stimulus, but responds to a broad band of wavelengths. This paper has added the observation that the colour-coded channels give sustained responses to flashed stimuli, while the broad-band channels give transient responses at the onset of the flash. Single neurones in the monkey visual system show a similar difference: colour-opponent neurones give sustained responses, whereas broad-band non-opponent neurones respond transiently[2–5]

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Solubilisation and partial purification of auxin-binding sites of corn membranes

THE demonstration by Hertel *et al.*[1] of reversible, high-affinity binding of auxins to membranous preparations from corn coleoptiles provided for the first time a reliable and readily accessible system for the study of potential receptor–phytohormone interactions. In a detailed investigation of the binding kinetics and specificity of this system, we presented evidence for the presence of two classes of auxin-binding sites with differing affinities and specificities[2,3]. Site 1, with a K_D for the synthetic auxin 1-naphthalene-acetic acid (NAA) of 0.14 μM, was able to bind both auxins and physiologically inactive analogues, whereas site (K_D for NAA = 1.7 μM) displayed binding specificity compatible with that expected of an auxin receptor[2] and was located in a heavier membrane population, enriched in plasma membrane[3]. Auxin-binding activity can be readily solubilised from the membranes with Triton X-100 (ref. 2), but our attempts to purify such extracts further have met with only limited success. I report here that both binding sites can be conveniently removed from the membranes without detergent and purified approximately 100-fold in two steps.

Membranes were prepared from 40–80 g of corn coleoptiles (*Zea mays*, Kelvedon 33) by differential centrifugation between 4,000*g* and 80,000*g*, as before[2]. The pellets were suspended using a Teflon–glass homogeniser, in 0.1 volume (based on original tissue weight) of grinding buffer[2] and the suspension was added to 20 times its volume of Analar acetone at 4 °C, with stirring. The clumped precipitate was separated from the yellow, opalescent supernatant by vacuum filtration or by low-speed centrifugation, washed with acetone and dried in a stream of nitrogen. The dried residue was extracted by homogenisation in DBB (a fivefold dilution of binding buffer, see Fig. 1 legend and ref. 2) and insoluble material was removed by centrifugation at 38,000*g*, 15 min.

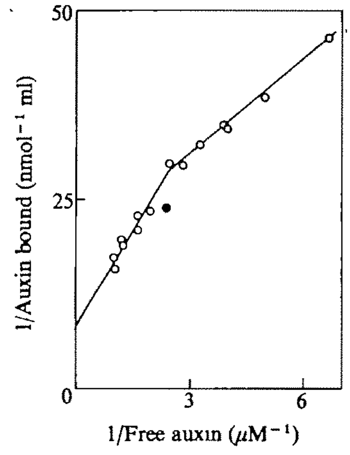

Fig. 1 Double reciprocal analysis of NAA binding to the crude membrane extract Samples of 1 ml (each equivalent to 2 g of tissue) were dialysed against 1 ml of ^{14}C-NAA (61 mCi mmol^{-1}, Radiochemical Centre, Amersham) in binding buffer (0 25 M sucrose, 5 mM $MgSO_4$, 10 mM citrate–acetate, *p*H 5 5), supplemented with varying amounts of unlabelled NAA to give a range of equilibrium concentrations from 0 15 to 1.0 μM. Equilibrium required a minimum of 5-h dialysis at 4 °C using Dianorm cells (MSE, Crawley) Radioactivity in 750-μl aliquots from each half-cell was determined by liquid scintillation counting The regression lines of the double reciprocal plot were fitted by the method of Wilkinson[4]; site binding constants were derived as described by Hunston[5] for a two-site system $K_1 = 0\ 17\ \mu M$, $n_1 = 14\ 3$ pmol g^{-1}; $K_2 = 1.4\ \mu M$, $n_2 = 51$ pmol g^{-1}.

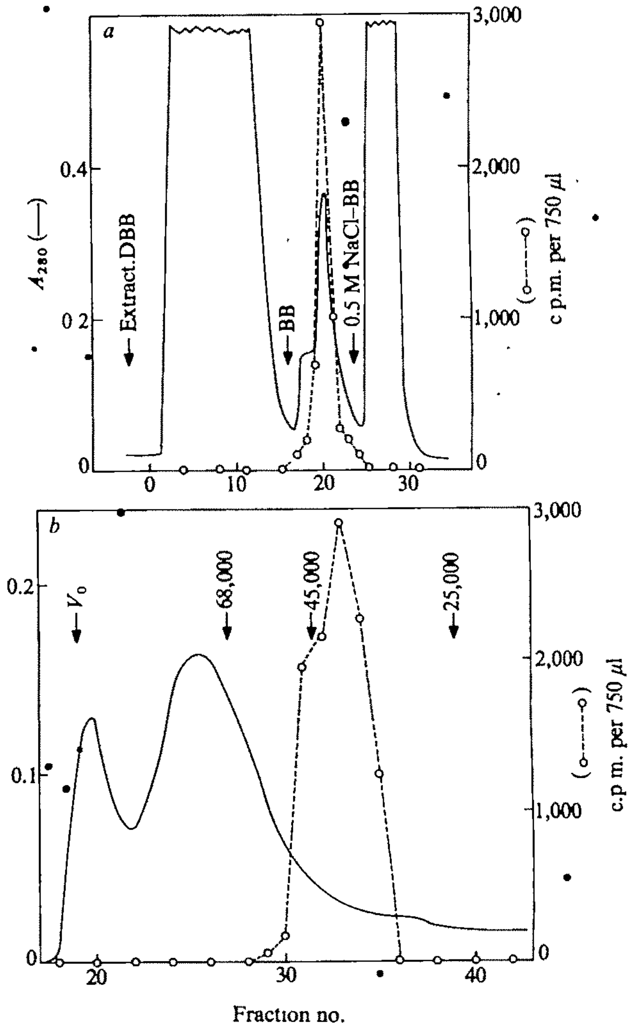

Fig. 2 Purification of auxin-binding proteins. The crude membrane extract was applied to a 5 × 1 5-cm column of DEAE Bio-Gel (Bio-Rad) equilibrated in DBB (fivefold diluted binding buffer; see legend to Fig 1) The column was washed with DBB until A_{280} approached the baseline value Elution was then continued step-wise with binding buffer (BB), followed by 0.5 M NaCl in binding buffer Fractions of 2.5 ml were collected and aliquots (diluted to 1 ml with binding buffer) were assayed for auxin binding by equilibrium dialysis against 1 ml of 0.3 μM ^{14}C-NAA Binding was determined from the radioactivity difference between each pair of half-cells The fractions with binding activity were pooled, concentrated to 1.8 ml by centrifugal ultrafiltration (Amicon CF25 membrane cones) and applied to a column, 81 × 1 5 cm of Sephadex G100 (Pharmacia), equilibrated in binding buffer Fractions of 2 0 ml were collected and assayed for auxin binding *a*, DEAE Bio-Gel profile, *b*, Sephadex G100 profile V_0, void volume (ferritin). Arrows indicate elution peaks of standard proteins of the molecular weights indicated. ———, Absorbance at 280 nm, — — —, ^{14}C-NAA binding

Binding of ^{14}C-NAA to these crude membrane extracts was examined by equilibrium dialysis Double reciprocal analysis (1/bound against 1/free) of the binding data yields biphasic plots (Fig. 1) similar to those obtained with intact membrane preparations[2], and suggesting that both sets of binding sites have been solubilised from the membranes. The dissociation constants ($K_1 = 0.17\ \mu M$; $K_2 = 1\ 4\ \mu M$) and the proportionality of binding sites ($n_1 : n_2$) are in good agreement with the values for site 1 and site 2 respectively deduced in earlier membrane experiments[2] The total yield of soluble binding sites in different experiments has ranged from 45 to 90% of that expected in the original membranes, the source of this variability is not clear

When the buffer extracts were fractionated on DEAE Bio-Gel, auxin-binding activity was confined to the peak eluted with binding buffer (Fig. 2*a*) The auxin-binding material is heat labile (>90% loss of activity after 5 min at 90 °C), non-dialysable, and precipitates at 70% ammonium sulphate saturation. The post-DEAE binding fractions are positive to the Lowry reagent[6] and nucleic acids were undetectable by organic micro-phosphorus analysis[7] The binding species are therefore likely to be proteins The pooled post-DEAE binding fraction was further purified on Sephadex G100, yielding a single, usually asymmetrical auxin-binding peak (Fig.2*b*). The apparent molecular weight at the shoulder (fraction 31) is 47,300 and at the peak (fraction 33), 40,300 Overall purification in the two-column steps on the basis of c p m. bound per mg protein (mean of three experiments) is approximately 100-fold (crude extract = 1750, DEAE peak = 14,500, G100 peak = 171,800).

The asymmetric nature of the binding profile from Sephadex G100 could indicate a partial resolution of site 1 and site 2 proteins Gel electrophoretic analysis of the post-Sephadex binding fractions indicates only limited heterogeneity, but additional purification and scaling-up are needed to assign the binding protein bands with greater confidence. Investigation of the effects of potential affinity labels on auxin binding to isolated corn membranes has permitted the tentative assignment of several of the amino acid residues in the binding site environment[8]. The availability of the solubilised binding proteins and the use of radioactive affinity labels will facilitate further characterisation of the binding sites

The problem of establishing directly the significance of the binding sites in relation to the physiology of auxin action is more difficult to approach, but in principle it should be possible to incorporate the binding proteins in a synthetic membrane system, perhaps in conjunction with an ATPase, and look for a specifically auxin-mediated response such as proton pumping[9]

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Relationship between amount of Epstein–Barr virus-determined nuclear antigen per cell and number of EBV–DNA copies per cell

EPSTEIN–BARR virus-determined nuclear antigen (EBNA) can be detected in all EBV-DNA-carrying cells[1,2]. This includes Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) tumour biopsy cells' and EBV-carrying lymphoid lines[1-5] Of the known EBV-associated antigens only EBNA is regularly associated with the presence of viral genetic material, no matter whether the cell line produces virus or not[6,7]. The composition and function of EBNA are largely unknown. Some recent reports suggest that EBNA is a protein that binds to DNA *in vitro*[8,9], and is associated with chromosomes *in vivo* at least in part[1]. EBNA might be a virally determined or virally altered chromosomal protein of the non-histone type[9] It is tempting to speculate that EBNA has a role in EBV-induced cell transformation ("immortalisation"[10]) and/or in the control of viral gene expression There is a wide variation in the average number of EBV genome equivalents between different EBV–DNA-carrying cell lines. In virus non-producer cell lines this number is a better approximation of the true EBV genome number for individual cells than in virus producer lines, and is relatively stable over long periods of serial passage[11] The EBNA content of EBV-containing cell lines also seems to vary between different cell lines, as judged visually in the fluorescence microscope We have measured the amount of EBNA per cell, by quantitative immunofluorimetry in different cell lines, in parallel with determining the average number of EBV–DNA copies, and established a correlation between these two parameters.

Eleven non-producer lines were tested Three were derived from Burkitt's lymphoma (Raji[12], Namalwa[13], Rael[11]), three were independently established somatic cell hybrids of Raji and Namalwa (RN2, RN17, RN21) (ref. 15), four were EBV-carrying lymphoblastoid cell lines of non-neoplastic origin (303L (ref. 16), 6410 (ref. 17), F265 (ref. 18), PESS (ref. 14) and one was an EBV-converted derivative of the EBV-negative BL line Ramos (AW-Ramos (ref 19)). The average number of EBV genome equivalents per cell was estimated by nucleic acid hybridisation as described previously[3]. Briefly, 10-μg aliquots of cellular DNA, immobilised on nitrocellulose filters, were incubated for 96 h at 45 °C with 1 ng (1.5×10^5 c.p.m.) ^{32}P-labelled EBV complementary RNA (cRNA) in 0.3 ml 0.9 M NaCl–0 09 M trisodium citrate, containing 50% formamide Negative (calf thymus DNA) and positive (NC37 (ref. 18) cellular DNA) controls were included in all experiments. The number of EBV genome equivalents per cell were calculated from the assumption that the molecular weight of EBV DNA is 10^8 (ref. 20), that of DNA from a diploid human cell 4×10^{12}, and that of DNA from a tetraploid cell 8×10^{12}.

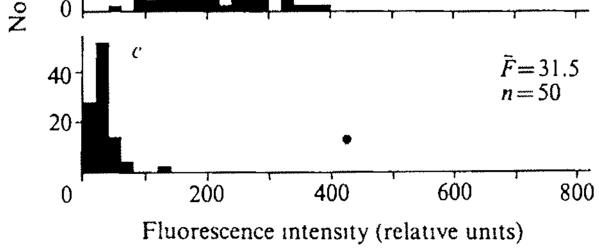

Fig. 1 Frequency distribution of EBNA fluorescence intensities of individual cells in cell lines that carry on average 160 (RN17, *a*), 60 (Raji, *b*) and 3 (Namalwa, *c*) EBV genome equivalents per cell

For immunofluorimetry, small circles were drawn on a slide with a diamond. Cells of different cell lines were placed within different circles and diluted to allow a distance of 2–3 cell diameters between individual cells, in order to facilitate individual cell measurement without neighbour interference. Two

Raji cell samples were placed on each slide for reference. The cell patches were dried, fixed in acetone–methanol (2:1) for 3 min and incubated with an EBNA-positive serum from a BL patient (M.A., Kenya Cancer Council No. 1530, EBNA titre 1:160, VCA titre 1:640 and EA-R titre 1:40, diluted 1:10 with BSS) for 30 min. Thereafter the slides were incubated for 30 min with 1:10 diluted complement from a healthy human donor (I.E.) with no antibodies to EBNA or to any other EBV-determined antigens. Staining was carried out using fluorescein-labelled goat anti-human β_{1c} (Hyland laboratories, Los Angeles, California). Fluorescence emission of randomly selected individual cells was determined by microspectrofluorimetry as described previously[21].

Figure 1 shows the frequency distribution of fluorescence intensity (FI) per cell in three lines. Raji and Namalwa were compared with one of their somatic hybrid derivatives, RN17 In each line fluorescence intensity per cell is distributed over a wide range. It is noteworthy that the FI values of the RN17 hybrid were not only higher than for either parent, but exceeded for most cells the additive value of the two parental lines.

EBNA fluorescence intensities of the same cell line (Raji) varied considerably between different experiments. To standardise the system, the mean value of the Raji cell patch on each slide was taken as reference value for the other lines measured on the same slide. This was chosen as a reference because the variation between Raji patches on the same slide was much smaller than between different slides and different experiments. Following this procedure the EBNA content of the different cell lines could be compared and studied in relation to the number of EBV genome equivalents per cell. As shown in Fig. 2, there was a strong correlation between these two parameters (*r* — 0.86). Three lines, Namalwa, 6410 and AW-Ramos, had low numbers of EBV genome equivalents per cell and they also had the lowest quantity of EBNA per cell. Four lines, 303L, F265, PESS, and Raji, had intermediate EBNA and EBV–DNA values. The three RN hybrids confirmed the impression gained from Fig. 1, in showing that the amount of EBNA per cell was not simply an addition of the parental values, but was amplified beyond the expected sum of the parents Even more interesting was the fact that this amplification seemed to be directly proportional to the amplified EBV genome copy number, already demonstrated by Andersson[11] One cell line shows comparably low fluorescence intensity, Rael. It had a very low EBNA value per cell, but a somewhat higher EBV–DNA copy number than expected. In this context it is interesting to note that Brown *et al*[22]. found that the binding of radiolabelled anti-EBNA antibodies to Raji nuclei was completely inhibited by nuclear extracts from several EBNA-positive cell lines, but only partially inhibited by Rael extracts. This suggests that Rael might lack some antigenic subcomponent of EBNA. This would also explain the low fluorescence intensity of Rael cells.

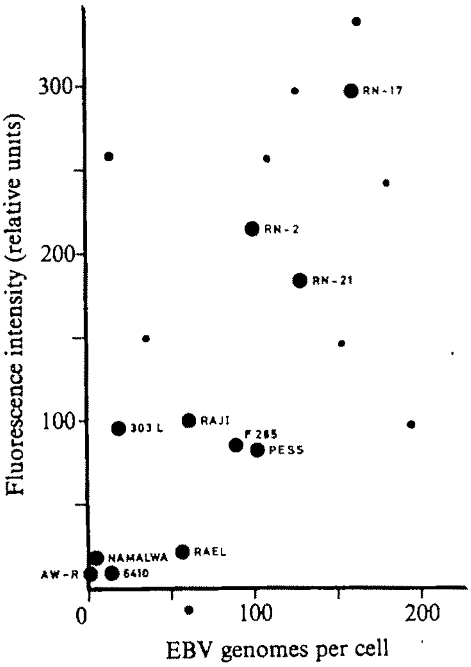

Fig. 2 Amount of EBNA per cell (mean cellular fluorescence intensity) plotted against average number of EBV genomes Mean EBNA fluorescence value of each Raji standard cell population has been normalised to 100. All individual cell values of the other lines were computed in relation to the reference value. The corrected mean values were entered for each cell line. These mean values are based on the following number of cells measured: Raji 358, AW-Ramos 162, Namalwa 143, PESS 56, F265 15, 6410 40, Rael 25, 303L 13, RN-2 40, RN-17 40, and RN-21 40. The number of EBV genomes was estimated by filter nucleic acid hybridisation as described in the text.

Obviously, the calculation of mean EBV–DNA and EBNA values masks the previously demonstrated cycle-dependent variation of both parameters[23] (also I.E. and D.K., unpublished). The multiple EBV genomes carried in non-producer Raji cells were shown to replicate during early S phase[23]. We have found that EBNA increases continuously during the cell cycle, most of the increase occurring during the G1 phase (I.E. and D.K., unpublished). The fact that the mean values show a strong correlation suggests that physiological variations tend to 'even out' in large populations sampled on different occasions

The relationship between mean EBV–DNA and EBNA values raises some intriguing questions. It is known that the 'immortalising', proliferative interaction of EBV with its host cell, the human or primate B lymphocyte, is entirely dependent on the restrictions of viral gene expression that prevent the entry of EBV-DNA-carrying cells into the invariably lethal viral cycle. Non-histone chromosomal proteins are believed to have a transcription-regulating role in differentiated cells[24,25]. Since EBNA seems to be a virally induced or virally changed chromosomal protein, it is tempting to speculate that it may have an analogous role, perhaps in direct relation to the EBV cycle. A positive correlation might be expected between EBNA and EBV–DNA if EBNA regulates the transcription of the multiple viral genomes. Alternatively, or in addition, EBNA may have a role in transforming the normal B lymphocyte into an indefinitely proliferating, established cell line. As a third possibility EBNA might be simply a product of the latent viral genomes, without having any role in viral transformation or in the maintenance of viral latency. The correlation we have found might then be due to a viral gene-dosage effect.

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Proton pump coupled to cytochrome *c* oxidase in mitochondria

CYTOCHROME *c* oxidase is an important enzyme of aerobic metabolism, catalysing electron transport between cytochrome *c* and molecular oxygen. Apart from this essential redox reaction the enzyme must be involved in conservation of the released redox energy for utilisation in ATP synthesis at the socalled third site of oxidative phosphorylation. I report here that the redox activity of mitochondrial cytochrome *c* oxidase is coupled to the translocation of hydrogen ions across the inner mitochondrial membrane This finding may be a clue to the role of cytochrome oxidase in energy conservation.

Cytochrome oxidase is located in the inner mitochondrial membrane, as are the other components of the respiratory chain and the ATP synthetase complex. Moreover, the enzyme seems to span the membrane, being exposed to some extent on both surfaces[1] Mitchell's chemiosmotic hypothesis of oxidative phosphorylation[2–4] postulates that cytochrome oxidase catalyses transmembrane electron transfer from cytochrome *c* (on the outside) to oxygen, which is reduced on the inside of the membrane with consumption of H^+ ions The vectorial electron transfer is suggested to give rise to a membrane potential ($\Delta\psi$) while the inside H^+ consumption gives rise to a *p*H gradient (ΔpH). The resulting electrochemical proton gradient (or protonmotive force, $\Delta\mu_{H^+} = \Delta\psi - 60\Delta p\mathrm{H}$) is suggested to be the driving force for oxidative phosphorylation[2–4].

The conformation of the haems in mitochondrial cytochrome oxidase changes in parallel to $\Delta\mu_{H^+}$, but does not correlate with either of its components, $\Delta\psi$ or ΔpH, alone[5]. Energisation of mitochondria, which is associated with the development of a protonmotive force of some 200 mV (positive outside)[6], shifts the enzyme into a more low-spin conformation with associated changes in spectral, redox and kinetic properties[5,7–11]

The parallelism between the electrochemical proton gradient and the conformation of cytochrome oxidase is demonstrated in Fig. 1, where a diffusion potential of potassium ions is specifically induced across the mitochondrial membrane with the aid of the ionophore valinomycin. As demonstrated previously[12], the electrical potential difference can be monitored spectrophotometrically using safranine as a probe, while ΔpH can be recorded by means of the *p*H indicator neutral red[13]. As Fig. 1 shows, the electrical diffusion potential decays slowly, as expected, from a membrane system orders of magnitude less permeable to ionic species other than potassium. Neutral red shows progressively more acidity inside the mitochondria, with kinetics lagging behind the formation of the electrical potential. This suggests a relatively slow flux of protons into the mitochondria in response to the induced membrane potential. The spectral shift in ferric cytochrome oxidase (see refs 5,7–10) develops with kinetics similar to the safranine signal, but decays with much faster kinetics, similar to those of neutral red. Thus the conformation of cytochrome oxidase follows neither the $\Delta\psi$ nor the ΔpH component of the protonmotive force, but seems to change in parallel with their sum ($\Delta\mu_{H^+}$), decaying as the protonmotive force decays primarily due to increased internal acidity.

Cytochrome oxidase would be expected to be sensitive to the electrochemical proton gradient if the conformational change of the enzyme were coupled to the translocation of hydrogen ions across the membrane. Thus $\Delta\mu_{H^+}$ may drive protons into the mitochondria through a 'pump' coupled to the high- to low-spin transition in ferric haem Conversely, and more important, redox activity of cytochrome oxidase may lead to the low-spin 'high energy' form of the enzyme, followed by transition to high-spin coupled to translocation of H^+ ions out across the membrane[14]

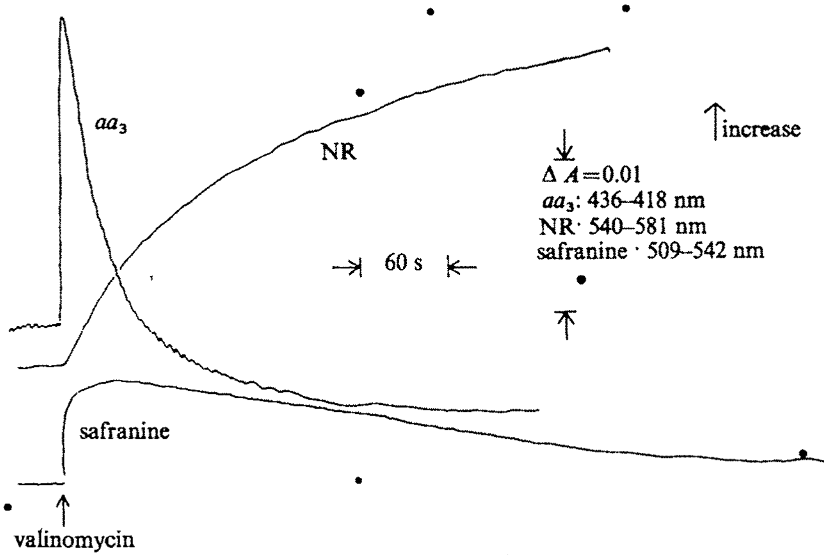

Fig. 1 Kinetics of membrane potential, *p*H gradient and spectral shift in ferric cytochrome *c* oxidase The reaction mixture contained 0.2 M sucrose and 30 mM HEPES buffer, *p*H 7.2, with further additions of 10 μM rotenone, 0.33 mM EDTA, 0.9 mM sodium ferricyanide, oligomycin (9 μg ml^{-1}) and rat-liver mitochondria (3.6 mg protein per ml) In the experiment marked NR, a further 45 μM of neutral red was added, and in the experiment marked safranine, this dye was added to a concentration of 20 μM Dual wavelength spectrophotometry was performed at the indicated wavelength couples, using cuvettes with 1.0-cm light path Temperature was 23 °C Each experiment was started after 30 s pre-incubation with the mitochondria, by addition of valinomycin (0.1 μg ml^{-1}). An upward deflection of the safranine trace indicates formation of an electrical membrane potential with positive polarity extramitochondrially[12] An upward deflection of the NR trace indicates acidification of the intramitochondrial space[13] (at the high extramitochondrial buffering power used the dye measures intramitochondrial *p*H only[13]) Upward deflection of the trace marked aa_3 signifies the energy-linked spectral shift in the ferric cytochrome *c* oxidase[5,8–10]

This possibility was tested by pulsing aerobic mitochondria with

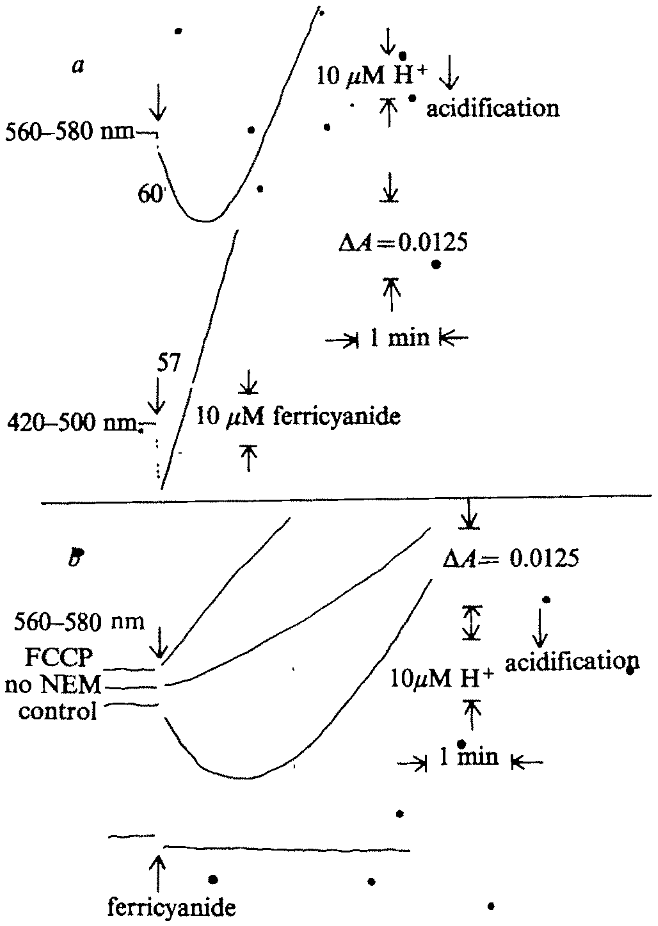

Fig. 2 Ejection of hydrogen ions coupled to oxidation of ferrocyanide in mitochondria. The reaction medium, 0.15 M KCl–1 mM HEPES, *p*H 7.4, was supplemented with 10 μM rotenone, antimycin (0.21 μg ml^{-1}), valinomycin (0.67 μg ml^{-1}), oligomycin (6.7 μg ml^{-1}), 0.67 mM EDTA, 0.33 mM NEM and rat-liver mitochondria[20] to a final concentration of 5.3 (*a*) or 6.0 (*b*) mg protein per ml. A dual wavelength spectrophotometer (Johnson Research Foundation Workshops, Philadelphia) was used at the wavelength couples indicated. An increase in the absorption difference results in an upward deflection of the trace. Light path was 1.0 cm and temperature was 23 °C. Recording of extramitochondrial *p*H (*a*, upper trace, and *b*) was done in the presence of 10 μM phenol red. The signal was calibrated with aliquots of HCl and found to be linear with (H$^+$) in the range measured. Ferricyanide production (*a*, lower trace) was recorded in the absence of the *p*H indicator. The signal was calibrated with known concentrations of ferricyanide. The reaction was started after 10 min preincubation by addition of 0.67 mM (*a*) or 0.33 mM (*b*, upper traces) ferrocyanide (at arrow). For the lower trace of (*b*), 0.67 mM ferricyanide was added as a control. Numbers adjacent to traces in (*a*) refer to initial rates in μM min^{-1} of H$^+$ production (upper trace) and ferricyanide formation (lower trace) respectively. In (*b*) 0.8 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was present when indicated.

ferrocyanide as electron donor. The mitochondria were pretreated with rotenone and antimycin to minimise endogenous respiration, and oligomycin to abolish any proton influx linked to phosphorylation of endogenous ADP. The experiments were performed in a KCl medium with valinomycin to ensure that any electrogenic proton efflux would be balanced electrically by influx of potassium ions. *N*-ethyl-maleimide (NEM) was further added to inhibit the proton–phosphate symporter (or hydroxyl–phosphate antiporter) of the mitochondrial membrane[15].

In these conditions oxidation of ferrocyanide by oxygen through cytochrome *c* and cytochrome oxidase indeed resulted in net release of protons into the extramitochondrial phase (Fig. 2*a*). The stoichiometry of the initial phase of the reaction was very closely one H$^+$ ion released per oxidised ferrocyanide, with ferrocyanide pulses between 0.13 and 1.35 mM. After the initial proton release there is alkalinisation of the mitochondrial suspension (Fig. 2) due to consumption of protons in the reduction of O$_2$ to water. In the presence of a proton-conducting uncoupling agent (FCCP, Fig. 2*b*), only the latter H$^+$ consumption is observed with the expected H$^+$/e$^-$ stoichiometry of 1.0. Fig. 2*b* also shows that inhibition of the proton–phosphate symporter by NEM is a prerequisite for observation of net H$^+$ ejection. Leaving out either the valinomycin or the oligomycin retarded net H$^+$ release (not shown). The lack of these constituents in the ferrocyanide pulse experiments of Mitchell and Moyle[16] may explain why they did not observe proton release. That the H$^+$ ejection is indeed coupled to oxidation of ferrocyanide and not to reduction of formed ferricyanide by endogenous substrates (though rotenone and antimycin were present to prevent this) was confirmed by the finding that ferricyanide itself produced negligible H$^+$ ejection (Fig. 2*b*). Moreover, inhibition of cytochrome oxidase by cyanide was fully inhibitory. Appropriate controls ensured that true *p*H changes were measured by the phenol red technique. Identical experiments using a *p*H electrode instead of the dye (not shown) confirmed that this was indeed the case.

It seems likely that the protons ejected into the extramitochondrial space originate in the mitochondrial matrix rather than in membrane protein components, for with large ferrocyanide pulses the number of protons ejected exceeds the content of respiratory chain complexes by a factor larger than 25. Moreover, with neutral red as an indicator of intramitochondrial *p*H (ref 13) a ferrocyanide pulse caused rapid alkalinisation (Fig. 3), which was highly inhibited by uncoupling agents, and the extent of which was enhanced by NEM.

These results suggest that the oxidation of ferrocytochrome *c* by oxygen as catalysed by mitochondrial cytochrome *c* oxidase is coupled to the activity of an electrogenic proton pump. The H$^+$ translocation is apparently linked to a conformational change in cytochrome *aa*$_3$ with an associated spin state transition in the haem structure. The basic mechanism thus resembles the Bohr effect in haemoglobin[17] though in cytochrome oxidase the protons are translocated across a membrane with conservation of energy. The

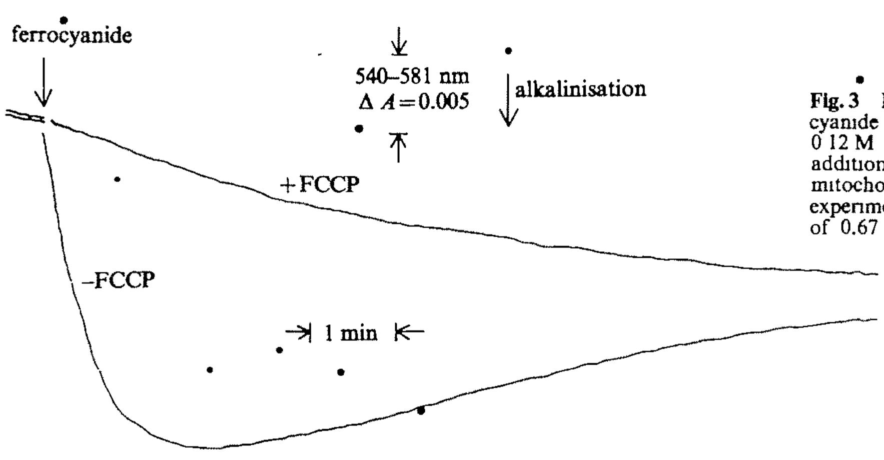

Fig. 3 Intramitochondrial alkalinisation coupled to ferrocyanide oxidation by mitochondria. Reaction mixture 0.12 M KCl–30 mM HEPES-Tris buffer, *p*H 7.4 with additions as listed in the legend to Fig. 2. Rat-liver mitochondria 5.2 mg protein ml^{-1}. Neutral Red 67 μM. The experiment was performed as described in Fig. 1, by addition of 0.67 mM ferrocyanide in the absence (−FCCP) or presence (+FCCP) of 0.8 μM FCCP.

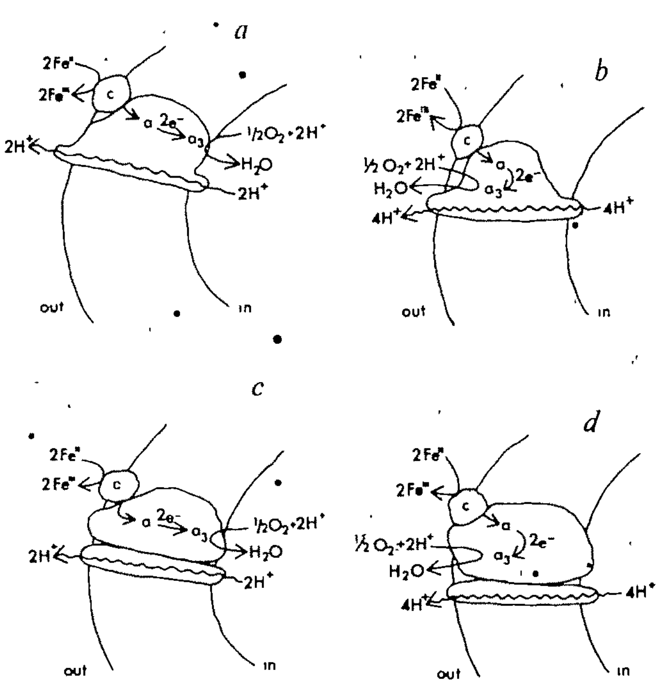

Fig. 4 Possible arrangements of the proton pump coupled to mitochondrial cytochrome c oxidase Symbols c, a and a_3 indicate the respective cytochromes, Fe^{II} and Fe^{III} indicate ferro-and ferricyanide respectively. For further details, see text.

observed H^+/e^- stoichiometry of 1 means that transfer of two electrons to $1/2\ O_2$ leads to separation of four charges across the mitochondrial membrane and not two as envisaged in the chemiosmotic hypothesis[18] As shown in Fig. 4, however, there are at least four possible arrangements of cytochrome oxidase and the proton pump in the mitochondrial membrane. Alternatives *a* and *c* retain the transmembrane electron transfer feature of cytochrome oxidase[4] but add a proton pump with a stoichiometry of 1 H^+/e^- either as part of (the subunit structure of) the enzyme itself (*a*), or as a separate molecular entity (*c*). If electron transfer is not electrogenic (*b* and *d*), the proton pump must be responsible for the entire charge separation and should thus operate with a stoichiometry of $2H^+/e^-$ (or $4H^+/2e^-$) to fit the present data Distinction between alternatives *a* and *b* compared with *c* and *d* should be possible by studying H^+ translocation in liposomes incorporated with purified cytochrome oxidase[19] Studies of this kind have shown (my and H T. Saari's unpublished work) that the proton pump described is an intrinsic property of this enzyme (*a* and *b*, Fig 4) rather than a separate molecular entity (*c* and *d*, Fig 4). A distinction between alternatives *a* and *c* compared with *b* and *d* may, however, be more difficult experimentally.

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Non-allelic variants of histones 2a, 2b and 3 in mammals

THE genome of eukaryotes is organised in nucleoprotein fibres formed by the interaction of the DNA with small basic proteins, the histones, and by specific interactions between certain histones[1] To gain more insight into the molecular organisation of chromosomes it is necessary to determine in detail the complexity and variability of the histones. The chromosomes of eukaryotes contain five major types of histones. One of these, the very lysine-rich H1, consists of a small number of polypeptides which differ slightly in primary structure and which are present in different relative amounts in different tissues[2]. The other four histones, which are involved in histone–histone interactions, were considered homogeneous and invariable until it became possible to resolve H2a, H2b and H3 into variants which exhibit tissue-specific variation by polyacrylamide gel electrophoresis in presence of non-ionic detergents[3] (Fig. 1). We report here on the primary structure of the variants of mammalian histones 2a, 2b and 3.

Individual histone components were isolated from calf thymus and several other mammalian tissues by preparative polyacrylamide gel electrophoresis (in the presence of Triton X-100) of fractions enriched in individual histone classes by chemical fractionation[4] or by ion exchange chromatography on CM-cellulose (S.G F. and A.Z. in preparation). Direct comparison of the amino acid compositions of the electrophoretically pure variants of the different histone classes indicated the presence of one to three amino acid substitutions in each case[5]. To determine the molecular differences between the variants in more detail, we analysed their tryptic or thermolytic peptides resolved by microfingerprinting on microcrystalline cellulose plates.

After visualisation by spraying with 10% triethylamine and 0.01% fluorescamine in acetone[6–7], the peptides (or in some cases only non-identical peptides) were eluted from the plates, hydrolysed and subjected to amino acid analysis. The position within the protein of the eluted peptides was determined by homology in amino acid composition with the published amino acid sequences for the major components of these histones in calf thymus[8–10] With the exception of the core of H3.3, no ambiguities were encountered in localising substitution sites by composition differences. The substitutions determined by these methods agree with the differences in composition found by amino acid analysis of the intact proteins.

The tryptic peptide maps of calf H2a.1 and H2a.2 were identical with the exception of one additional spot seen in H2a.2. This spot was found to be Ser-Arg (actual analysis: serine, 1.94; arginine, 1.06) arising from a serine–threonine substitution at position 16, known also to occur in the rat[11]. All other tryptic peptides from H2a.2 were homologous to H2a.1 peptides. Thermolytic digestion of the insoluble tryptic core of H2a.2 produced a peptide homologous with residues 43–51 (proline, 1.16; glycine, 2.25; alanine, 2.11; valine, 2.01; methionine, 0.53; tyrosine, 0.92) except that a methionine residue was present and leucine 51 was absent. Cleavage of H2a.2 with cyanogen bromide, isolation of the fragments by preparative polyacrylamide gel electrophoresis and amino acid analysis confirmed this methionine–leucine substitution at position 51 and the serine–threonine substitution at position 16.

Both tryptic and thermolytic peptide maps of mouse ascites H2b.1 and H2b.2 were identical. Since differences in hydrophobicity determine the resolution of histones in non-ionic detergent electrophoresis, we examined the hydrophobic regions. Peptide T12 (ref. 12) was homologous with residues 73–79 except that in H2b.2 it contained an additional serine residue and no glycine (serine, 1.79; glutamic acid, 1.39; alanine, 2.18; isoleucine, 1.05; arginine, 0.59). This indicates a serine-glycine substitution at position 75. Calf thymus and other mammalian tissues contain a minor component which migrates similarly to H2b.2 on electrophoresis. The tryptic peptide map of this minor component from calf thymus is identical to those of mouse and calf H2b.1 except that it possesses one extra spot. The composition of this spot (serine, 1.23; glutamic acid, 1.19; glycine, 0.93; alanine, 1.83; isoleucine, 0.85; arginine, 0.96) is identical with T12 (which is also present on the map) of H2b.1 yet it moves considerably farther in the electrophoretic and chromatographic dimensions. The most likely explanation for this is the substitution of a glutamine for a glutamic acid at position 76 in a new H2b variant, H2b.3. This is consistent with its amino acid composition being identical to H2b.1. The simultaneous presence of peptide T12 in two spots is probably due to partial deamidation of glutamine 76.

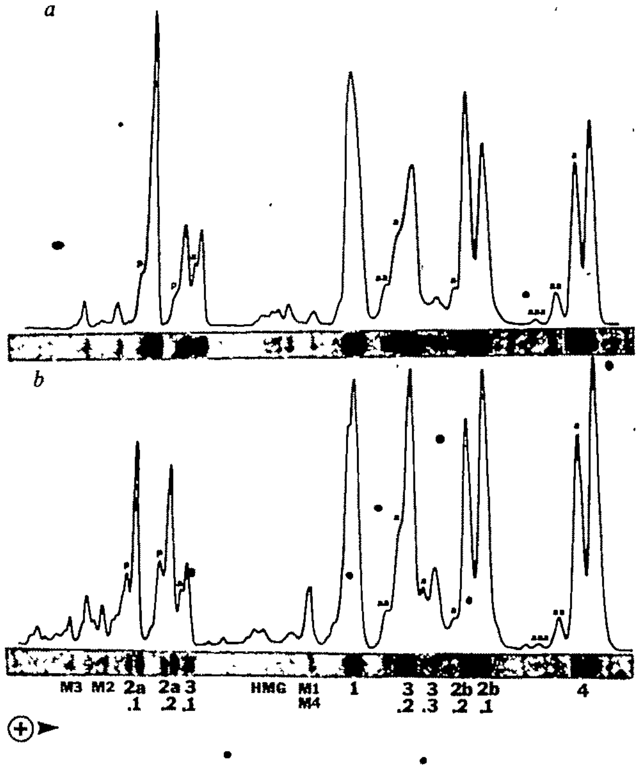

Fig. 1 Comparison of the histones of mouse thymus (*a*) and mouse liver (*b*) as resolved by electrophoresis (from left to right) on 12% polyacrylamide gels containing 7.5 M urea, 6 mM Triton X-100 and 5% acetic acid. The proteins were stained with amido black and the gel scanned at 300 nm. 1, 2a, 2b, 3, and 4 = major histone species; .1, .2, .3 = variants of a given histone species; M1, M2, M3, M4 = minor histone species with properties characteristic for histones but amino acid compositions not closely related to any of the five major histone species[13]; HMG = 'high mobility group' proteins[27]; a = acetylated, p = phosphorylated forms. Note the difference in the relative amounts of H2a.1 against H2a.2, H2b.1 against H2b.2, H3.1 against H3.2 and H3.3 in the two tissues.

Tryptic maps of calf H3.1 and H3.2 are identical but the thermolytic map of H3.2 shows a major spot in a region where H3.1 shows a cluster of faint spots. Anlaysis of the H3.2 spot revealed a peptide homologous with residues 92–99 of H3.1 except that it contained no cysteine and had one serine (serine, 0.92; glutamic acid, 3.06; alanine, 2.11; leucine, 0.93; tyrosine, 0.97). Thus a serine–cysteine substitution exists at position 96, in agreement with the findings of Patthy and Smith[13] and known to occur also in shark, carp and chicken[14–16]. From thermolytic digestion of the tryptic cores of calf and sheep H3.3 we have confirmed that it also contains the above serine–cysteine substitution. The remainder of the core peptides are homologous with the sequence of H3.1 except that we cannot find a peptide corresponding to residues 88–90 (Ala-Val-Met). Instead we recover a peptide with the composition: alanine, 1.03; isoleucine, 0.90; glycine, 1.07. The most likely substitution pattern indicated by our data would be: isoleucine instead of valine 89 and glycine instead of methionine 90 The latter substitution is not conservative and occurs rarely[18]. Pea H3, however, also contains a 'helix-breaker' (serine) at position 90 (ref. 17). The arrangement of amino acids in this region of H3.3 has to be confirmed by direct sequence studies.

Figure 2 summarises our findings for all the mammalian H2a, H2b and H3 variants which we have characterised so far. All the substitutions (except for position 16 in H2a) are located in hydrophobic regions where the 'helix probabilty'[19–20] is sensitive to small changes in amino acid side chains All but one of the substitutions are conservative and result in small changes in the detergent affinity except for the cysteine–serine substitution in H3 which has a large effect on detergent affinity. This is consistent with the observed general correlation between detergent affinity and helix probability[2,21], since, although cysteine and serine are similar in configuration, they belong to different categories of helix promoting ability[19]. Our findings for the histone variants demonstrate the extreme sensitivity of our method to small differences in hydrophobic properties of proteins, which facilitates the separation of proteins differing in a single conservative amino acid substitution involving neutral amino acid residues.

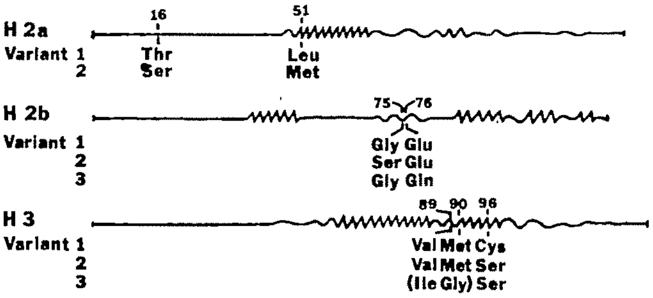

Fig. 2 Substitution sites in the mammalian histone variants. The degree of high helix probability[20] in different regions of the proteins are symbolically indicated by wavy lines

Except for H2b.2, which has only been found in the mouse[22], all variants have been found simultaneously in all tissues of all mammals examined, although in different relative amounts[23]. The simultaneous presence of all variants in plasmacytomas and tissue culture lines derived from inbred mice, indicates that the genes for the different histones are non-allelic. Furthermore, all variants are synthesised simultaneously in HeLa cells[24] from mRNAs with slightly different properties[24]. In the sea urchin, different H2a and H2b variants are synthesised at different stages of embryonic development which suggests the existence of different histone operons[25,26].

These observations, together with the parallel conservation of the non-allelic histone variants throughout the evolution of at least the mammals, suggest that the histone variants have independent essential functions. The histone classes H2a, H2b, H3 and H4 interact by non-ionic forces to form highly ordered heteropolymers which constitute the central structural element of eukaryotic chromosomes[28]. The amino acid substitutions distinguishing the histone variants are located in the hydrophobic regions which are involved in these histone–histone interactions. It is therefore conceivable that chromosomal fibres containing histone complexes composed of different histone variants differ significantly in their physical properties and that different histone variant populations are necessary for optimal function of specific regions of the genome in nuclei of cells with different metabolic patterns.

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Formation of amino acids from elemental carbon by contact glow discharge electrolysis

THE formation of amino acids in various inferred prebiotic conditions has been widely studied[1,2]. Recently we reported syntheses of amino acids from aliphatic carboxylic acids and ammonia[3,4], and from aliphatic amines and formic acid[4,5] by means of contact glow discharge electrolysis (CGDE)[6]. We describe here the formation of amino acids and urea from elemental carbon in the presence of aqueous ammonia by CGDE. By this reaction and the following process, the elemental carbon of the elctrode was converted into the bio-organic compounds.

The reaction tube used for CGDE was as illustrated in Fig. 1. The carbon rod (Irikawa, IBGC-55; diameter 5 mm; specific gravity 1.65, electrical resistance 250 Ω) was used as the anode and platinum wire was used as the cathode. All glassware used was heated for 1 h at 500 °C before CGDE to eliminate possible contamination of amino acids[7] Anhydrous ammonia was bubbled into the twice-distilled water (about 10 ml) in the reaction tube and then CGDE was performed for 35 min. The applied electric current was 60 mA at 600–1200 V. CGDE was conducted in conditions of ammonia gas saturation and the reaction temperature was kept at about 30 °C by cooling the reaction tube in a methanol–dry ice bath After the reaction was over, the solution was heated at 90 °C for 23 h in a sealed tube and was evaporated to almost dryness under reduced pressure. The residue was hydrolysed with 2 ml of distilled 6 N hydrochloric acid in a sealed tube under reduced pressure After heating at 120–130 °C for 23 h, the sealed tube was opened and the mixture evaporated to dryness under reduced pressure The residue in the tube was diluted appropriately and was analysed by an amino acid analyser (Yanagimoto LC-5S).

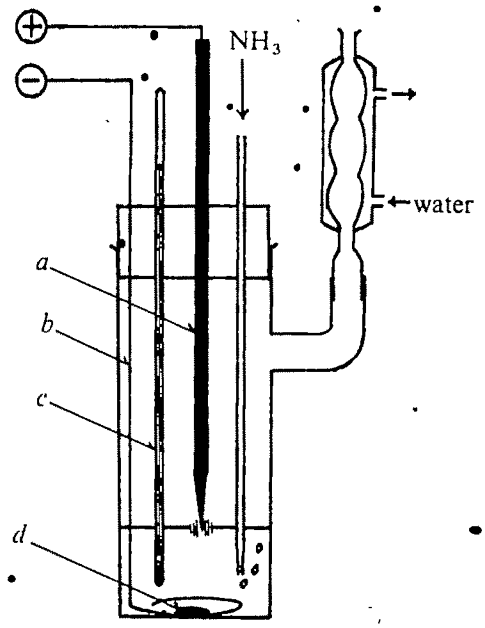

Fig. 1 Reaction tube for CGDE, *a*, carbon anode; *b*, platinum cathode; *c*, thermometer; *d*, magnetic stirrer bar

A typical result of the amino acid analysis is shown in Fig. 2*B*. Several control experiments were carried out without applying CGDE, and only trace amounts of glycine were detected (Fig. 2*A*). The weight loss of the carbon anode after the CGDE reaction was 5.6 mg. Thus the conversion of elemental carbon to organic carbon was at least 0.2% (during CGDE a very small amount of carbon

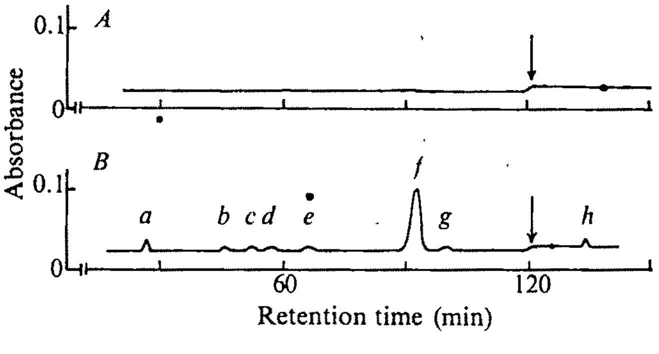

Fig. 2 Amino acid analysis *A* Control experiment without CGDE A trace amount of glycine (~10^{-10} mol) was detected The peak is not visible in the chart *A*. *B*, Reaction conditions were: electric current, 60 mA, reaction time 35 min Compounds found in the reaction mixture were *a*, urea (6 1 × 10^{-7} mol); *b*, aspartic acid (1 × 10^{-9} mol), *c*, threonine (1 × 10^{-9} mol), *d*, serine (8 × 10^{-10} mol), *e*, glutamic acid (5 × 10^{-10} mol), *f*, glycine (4 5 × 10^{-8} mol); *g*, alanine (1 × 10^{-9} mol); *h*, unknown peak Arrows indicate buffer change.

powder was found in the reaction mixture, so 0.2% is the minimum).

To our knowledge, this reaction is the first example in which amino acids and urea were obtained directly from elemental carbon These facts suggest a new possibility for the prebiotic synthesis of amino acids in prebiotic conditions on the primordial Earth.

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Enzyme hydration may explain catalytic efficiency differences among lactate dehydrogenase homologues

HOMOLOGOUS enzymes of species with different cell temperatures usually display temperature-adaptive differences in their catalytic properties[1–7]. Thus enzymes of low cell temperature species characteristically exhibit higher specific activities and lower activation free energies and enthalpies than the homologous enzymes of high cell temperature species[2–7]. For lactate dehydrogenase (LDH, E.C.1.1.1.27; lactate: NAD$^+$ oxidoreductase), a single M_4 (muscle type) LDH molecule from a cold-water fish can catalyse the conversion of pyruvate to lactate three times as rapidly at 5 °C as an M_4-LDH from a mammal[5]. In this paper we present evidence that these temperature-compensating differences in catalytic efficiency may derive in part from different amounts of exergonic hydration of the enzymes during the activation step in catalysis. Lowering the activation free energy ($\Delta G\ddagger$) 'barriers' to enzymic reactions by way of the reversible hydration of amino acid side chains and/or polar peptide linkages permits the adjustment of catalytic efficiencies without necessitating the modification of amino acid sequences in the active sites of the enzymes.

Attempts to explain mechanistically the differences in catalytic efficiency which exist among homologous forms of the same enzyme must face the following paradox: even though different homologues vary significantly in catalytic efficiency, the amino acid sequences of their active (catalytic) sites are virtually identical[8–10]. Where amino acid substitutions in active sites have occurred, they tend to be extremely conservative[8]. It therefore seemed reasonable to consider non-active site regions of the enzyme when attempting to explain the differences in catalytic efficiency among differently temperature-adapted LDH homologues.

Recently a rate-enhancement mechanism which is not based on active site conformations has been proposed, the 'protein group transfer–hydration mechanism'[11]. Rate enhancement by this mechanism results from the exergonic transfer of protein groups (amino acid side chains and peptide backbone linkages) between the hydrophobic interior of the enzyme and the enzyme–water interface during the activation step in catalysis. These exergonic transfers will lower the free energy of the activated complex, thereby enhancing its probability of formation and accelerating the reaction rate. For example, the transfer of a peptide linkage from the interior of the enzyme, where it is relatively or fully dehydrated, to the enzyme–water interface, where a strongly exergonic hydration of the group will occur, can lower $\Delta G\ddagger$ by as much as 1,100 cal mol^{-1} (ref. 11). Since changes in $\Delta G\ddagger$ of only 100–200 cal mol^{-1} are adequate to approximately double the rate of a reaction[2], even slight changes in the exposure (hydration) of protein groups could be sufficient to significantly modify the catalytic efficiency of an enzyme. Since conformational changes seem to have a generally important role in enzymic catalysis[12], numerous changes in protein group exposure to water will occur during the activation event Thus the protein group transfer–hydration mechanism of rate enhancement seems a generally accessible mechanism for rate enhancement of all types of enzymes.

In the present study we have tried to determine if different amounts of exergonic hydration processes during the activation step occur in the LDHs of warm- and cold-blooded vertebrates. Three experimental tests, all of which are based on the energy and volume changes which accompany protein group transfer–hydration processes, were carried out.

First, the transfer of all types of protein groups from a relatively non-polar environment, such as the enzyme interior, to water occurs with a volume decrease arising from the dense organisation of water around the hydrated protein group[13]. Thus a rough estimate of the involvement of protein group transfer–hydration processes in catalysis can be obtained from the volume change which occurs during the activation event. The activation volume ($\Delta V\ddagger$) equals the difference in system (enzyme plus solvent) volume between the ground-state enzyme–substrate complex and the activated complex[14]. The activation volume of a reaction can be determined by estimating the effect of hydrostatic pressure on the maximal velocity (V_{max}) of the reaction[14]. If the highly efficient LDHs of low cell temperature organisms become more hydrated during the activation step

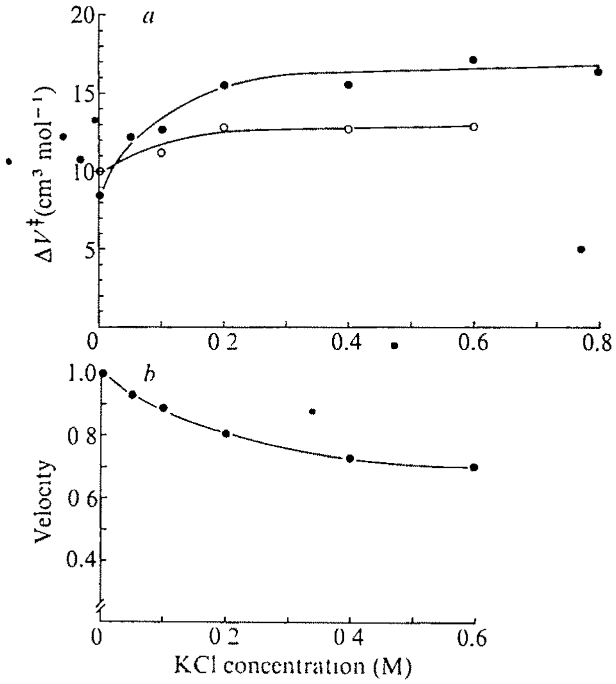

Fig. 1 *a*, Effect of KCl concentration on $\Delta V\ddagger$ of the halibut (●) and turkey LDH reactions (○). *b*, Effect of KCl concentration on V_{max} of the halibut LDH reaction. All assays were carried out using fully purified LDHs (Dr Nathan O. Kaplan). The assay medium contained, in a total volume of 5.0 ml, 80 mM Tris-HCl buffer (*p*H 7.5 at the assay temperature, 10 °C), 0 025 mM NADH, varying concentrations of pyruvate and KCl, and enzyme, which was added last to initiate the reaction The change in A_{340} was followed with time. The high pressure assay system was as described by Low and Somero[11,13]. The inhibition data were obtained from assays run at 1 atm Theoretical V_{max} values were determined using several pyruvate concentrations; data were analysed using plots of 1/velocity against 1/pyruvate concentration. For determining activation volumes the reactions were run at 1,000 and 5,000 pounds per square inch (68 and 340 atm, respectively). The logarithm of V_{max} for the different LDHs varied linearly with pressure over the pressure range of 1 to approximately 500 atm.

than the LDHs of birds and mammals, then a larger negative contribution to $\Delta V\ddagger$ will be found for the cold-adapted enzymes.

A second experimental test of this hypothesis is provided by the fact that the free energy changes which accompany these transfer processes are strongly influenced by the salt composition and concentration of the medium[15]. Salt conditions which make these transfers less exergonic would be expected to have two measurable effects. First, the velocity of the reaction will be reduced since the transfer will be energetically less favourable. Second, by impeding the hydration of transferred protein groups, salts will increase $\Delta V\ddagger$. Thus a comparative estimate of the parts played by protein group transfer–hydration processes can be obtained by measuring salt effects on V_{max} and $\Delta V\ddagger$. If these transfer processes are responsible for the higher specific activity of LDHs of low cell temperature species, then the salt sensitivities of V_{max} and $\Delta V\ddagger$ should be greater for the cold-adapted enzymes.

A third approach to analysing the parts played by these transfer–hydration processes in LDH homologues is based on the fact that these transfers occur with substantial enthalpy changes[16,17]. Also, as in the case of transfer free energies and volumes, transfer enthalpies will be affected by solute conditions[18]. Thus, if protein group transfers contribute also to the low $\Delta H\ddagger$ values of enzymes of cold-adapted organisms, then the $\Delta H\ddagger$ values of cold-adapted LDHs should be more salt-sensitive than the values of avian and mammalian LDHs.

The data presented in Figs 1 and 2 and in Table 1 support all of the predictions made above. Figure 1 illustrates the manner in which a weakly salting-out salt, KCl, affects the $\Delta V\ddagger$ and V_{max} of the LDH reactions. Increasing KCl concentrations titrate both the rate and $\Delta V\ddagger$, with essentially complete titration occurring at KCl concentrations of approximately 0.2–0.4 M. The V_{max} and $\Delta V\ddagger$ values of the halibut reaction are significantly more salt-sensitive than the values for the turkey LDH reaction (Fig. 1, Table 1), the result predicted on the basis of a greater degree of exergonic exposure and hydration of protein groups during the activation step in the halibut LDH reaction.

Table 1 lists the effects of 0.6 M KCl on V_{max} and $\Delta V\ddagger$ for LDH reactions of several warm- and cold-blooded species. The highly efficient teleost fish LDHs are significantly more inhibited than the other LDHs, and the salt-induced increase in $\Delta V\ddagger$ is also largest for the teleost enzymes. The shark LDH is intermediate in salt sensitivity between the teleost and avian–mammalian enzymes. Elasmobranch LDHs are also intermediate between teleost and avian–mammalian LDHs in catalytic efficiency[19].

Figure 2 illustrates the effects of 0.6 M KCl on the $\Delta H\ddagger$ values of the halibut and turkey LDH reactions. KCl has virtually no effect on the $\Delta H\ddagger$ of the turkey LDH reaction. $\Delta H\ddagger$ values of 10,600 and 10,200 cal mol^{-1} were observed in the absence and presence, respectively, of KCl. For the halibut reaction, addition of 0.6 M KCl to the assay medium increased $\Delta H\ddagger$ from 7,520 cal mol^{-1} to 9,260 cal mol^{-1}. Only approximately 2 cal mol^{-1} of this increase are due to changes in pressure–volume work ($\Delta H\ddagger = \Delta E\ddagger + P\Delta V\ddagger$) arising from the salt-induced increase in $\Delta V\ddagger$ (Table 1, Fig. 1). The Arrhenius plot for the halibut LDH reaction in the presence of 0.6 M KCl displays a 'break' near 25 °C. This discontinuity in slope may be the result of a salt-induced change in enzyme structure at higher temperatures.

The finding that the size of $\Delta V\ddagger$ at 0.6 M KCl is greatest for the teleost LDHs (Table 1) may be of importance in understanding the structural differences which exist among the LDHs. The $\Delta V\ddagger$ observed at KCl concentrations between approximately 0.2 and 1.0 M may be a rough measure of the change in protein void volume which arises from alterations in the packing efficiency of amino acid residues during the activation event[13]. That is, this KCl-insensitive component of $\Delta V\ddagger$ is a 'structural' activation volume as distinct from the 'hydration density' component of $\Delta V\ddagger$, which is due to water organisation around transferred protein groups and which is titrated by low KCl concentrations[13]. Within this conceptual framework the larger $\Delta V\ddagger$ at high salt concentration found for the teleost LDHs is interpreted as being due to a greater degree of enzyme unfolding during catalysis. This increased expansion of enzyme structure during catalysis by the teleost LDHs may be possible because of the smaller amounts of secondary structure in these enzymes relative to the LDHs of birds, mammals and elasmobranchs[19]. Thus, the more flexible LDHs of cold-adapted species may be capable of more fully unfolding their conformations to expose protein groups during catalysis. This interpretation is consistent with the finding that the thermal stabilities of enzymes are frequently inversely proportional to their catalytic efficiencies[3,4].

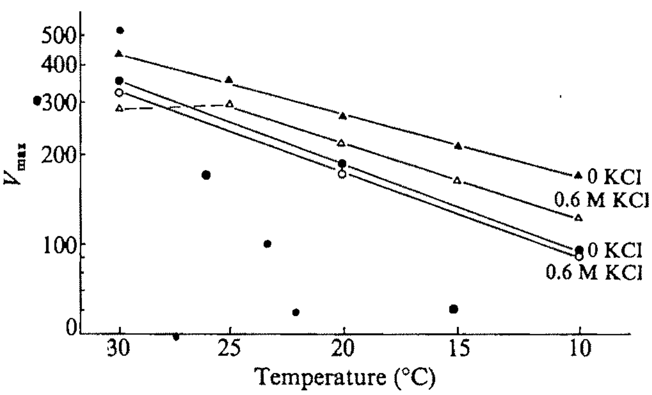

Fig. 2 Arrhenius plots showing the effect of 0.6 M KCl on the activation enthalpy value of the halibut (triangles) and turkey LDH reactions (circles). Assay conditions were as given in Fig. 1. Theoretical V_{max} values were determined using several concentrations of pyruvate. ▲, zero KCl; △, 0.6 M KCl; ●, zero KCl; ○, 0.6 M KCl.

In summary, the apparent necessity for maintaining the same amino acid sequences in both the binding and catalytic loop

Table 1 Effects of 0.6 M KCl on $\Delta V\ddagger$ and V_{max} for LDHs from warm- and cold-blooded organisms

Species	$\Delta V\ddagger$, no KCl*	$\Delta V\ddagger$, 0.6 M KCl*	600–0*	% Inhibition
Rabbit	9.9±0.7 (3)	12.3±0.5 (3)	2.4	11
Turkey	10.0±1.4 (6)	12.9±1.4 (6)	2.9	5
Shark (*Carcharodon carcharias*)	7.8±1.3 (7)	14.2±1.3 (6)	6.4	9
Tuna (*Thunnus thynnus*)	9.4±1.5 (9)	19.6±0 8 (9)	10.2	17
Halibut (*Hippoglossus stenolepis*)	8.5±0.9 (4)	16.0±1.4 (11)	7.5	29
Haddock (*Melanogrammus aeglefinus*)	8.4±1.4 (5)	17.2±2.3 (3)	8.8	20

*Unit: cm^3 mol^{-1}. Mean ±1 s.d. (sample size) for columns headed no KCl and 0.6 M KCl.

†Inhibition of V_{max} due to addition of 0.6 M KCl to the assay medium. Theoretical V_{max} values were determined using several pyruvate concentrations; data were analysed using plots of 1/velocity against 1/pyruvate concentration. Theoretical V_{max} values were used because KCl inhibited the reaction competitively as well as non-competitively.

regions of all homologues of M_4-LDH (ref. 8) does not hinder the functional adaptation of each LDH to the temperature conditions it experiences. For the LDHs of low cell temperature species, which must function at low and, in many cases, highly variable temperatures, adaptive reductions in $\Delta G\ddagger$ and $\Delta H\ddagger$ seem to have been achieved by means of increased exergonic exposures of hydrophilic protein groups during the activation step in catalysis. Thus, a higher catalytic efficiency (lower $\Delta G\ddagger$) and a reduced temperature dependency (lower $\Delta H\ddagger$) can both be achieved by evolutionary changes in enzyme structure at regions remote from the active site.

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Calculated forbidden band gap in periodic protein models indicating them to be insulators

THE idea of electron and hole transport between distant sites in biological macromolecules, especially in fibrous proteins[1] prompted quantum chemists to calculate the forbidden energy band gap in simple periodic protein models[2–7]. Conductivity measurements of several proteins under various conditions indicated a band gap or activation energy (E in the expression for the conductivity $\sigma = \sigma_0 \exp(-E/2kT)$ as function of the temperature T) of the order of 3 eV (refs 8, 9), a value in agreement with the only theoretical energy band gap available at that time (ref. 2). Therefore, the intrinsic mechanism of semiconduction along hydrogen bridges seemed to be well established in 1960. Later, however, both experimentally[10–13] and theoretically the question arose whether the conduction mechanism is really intrinsic. The more refined all-valence-electron quantum chemical calculations[3–7] indicated larger gaps (values in the range 6.1–16.7 eV were reported) than obtained in experiments. Most interestingly the calculation of Morokuma[5] at complete-neglect-of-differential-overlap level showed that in a polyglycine chain the bandgap was not too sensitive to the changes of the chain's conformation: in conformers with appreciable H-bonding the gap found was slightly larger than in the β-sheet conformation This latter finding pointed out the very small influence of H-bonds on the forbidden gap of periodic protein models. These semi-empirical calculation methods were designed for molecular purposes and there is still little known of their applicability to the calculation of the gap in crystals and polymers[14,19]. Thus we describe here use of the more reliable non-empirical ('*ab initio*') crystal orbital method[15] to calculate the band gap in a most simple polypeptide model β-polyglycine.

We used an STO-3G atomic orbital basis set[16]. After evaluation of all integrals[17], as usual in molecular *ab initio* calculations, we performed a self-consistent-field calculation using a first neighbour's interaction approximation. This highly sophisticated calculation resulted in a gap of 19.23 eV, the largest value obtained so far. Although larger numbers of included neighbours, basis set enlargement and correlation corrections may reduce this value by several eV, it seems impossible to reduce this value to the vicinity of the experimental gap. We may conclude therefore, that the intrinsic mechanism in a periodic protein model can be ruled out. Absolutely pure periodic proteins are thus good insulators. This conclusion will probably remain valid even in presence of orientational randomness of the constituent units. A set of aperiodic side chains, however, may serve as impurity centres, which support extrinsic type conduction.

The lowest energy optical transition of the electron system corresponds to the formation of excitons within the forbidden gap rather than to free electrons and holes. The order of magnitude of the energy of these excitons is expected to be 6–8 eV on the basis of the first electronic transition energies of small peptides[18]. Although this value is much larger than the experimental E it is necesarily smaller than the theoretical band-to-band gap found in our calculation.

Some technical details, which are of interest to specialists only, will be given elsewhere.

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Errata

The caption for the cover picture for *Nature*, vol. **264**, no. 5583 should have read : Microtubules polymerised *in vitro* and stained with the PAP-procedure with omission of the anti-tubulin antibody solution.

In the Statement published in *Nature*, vol **265**, p 764, the first line of the last paragraph of the letter from B Hamprecht should have read 'The data on cyclic GMP were' . . .

In the article 'Low temperature specific heat of glasses and amorphous solids' by P R Couchman, C L. Reynolds, Jr, & R. M J Cotterill, *Nature*, **264**, 534 (1976), the units for G in paragraph three are dyn cm^{-2}, not dyn cm^{-1}.

matters arising

Estimation of heritability from IQ data on twins

IN a recent paper[1] a nationally representative sample of twins born in one week of March 1958 was investigated to estimate kinship correlations in mental test performance for monozygotic and dizygotic twins. The authors concluded that their study offered "supportive evidence for zero or low upper limit heritabilities of mental test performance". We have received several critiques of the paper which we publish below, together with a response from the authors.

ADAMS *et al.*[1] have used a method[2] of estimating heritability, that is not reliable. The formula $h^2 = (r_{MZ} - r_{DZ})/(1 - \rho_{00})$ was stated by Jensen[2], without any theoretical justification, to measure heritability. In this formula r_{MZ} and r_{DZ} are the phenotypic correlations between monozygotic and dizygotic twins, respectively, and can be observed directly, but ρ_{00}, the genetic correlation between sibs, cannot be observed. It can be obtained from their phenotypic correlation if heritability is known, which, of course, it is not.

To overcome this difficulty, Jensen[2] suggests that the formula $\rho_{00} = (1+\rho_{pp})/(2+\rho_{pp})$, where ρ_{pp} is the genetic correlation between mates should be used to find ρ_{00}. He gives Li[3], chapter 13, as reference for this formula. However, it does not appear there, and Professor Li informs me (personal communication) that he had never seen it before I brought it to his notice. This formula seems to have no theoretical justification. Moreover, it does not resolve the problem of finding genetic correlation. We now require the genetic correlation between mates which can be obtained from their phenotypic correlation if heritability is known. Thus, to use Jensen's formula for finding heritability of a trait, an estimate of that heritability is required.

Jensen[2] resolves the dilemma by assuming that the genetic correlation between mates is 0.25, without telling his readers how this figure is obtained from their phenotypic correlation of 0.6 (ref. 4). The value of ρ_{DZ}, actually ρ_{00}, which Adams *et al.*[1] use is based on this assumed genetic correlation. The value of 0.8 for the broad heritability of IQ which is often quoted by Jensen and others is also based on this value. It is difficult to find a scientific justification for the method or to have any faith in estimates of heritability based on it.

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THE report by Adams *et al.*[1] is noncontributory to the estimate of heritability of mental test performance for three reasons:

(1) No adequate information is given on the zygosity of the twin pairs. With a small sample (<100 like sex pairs) there would have been little difficulty in testing the twins for genetic markers such as blood groups, or at least having the twins examined by an experienced human geneticist, with special tests on those difficult to assign. It is true that a competent questionnaire to the parents will enable the correct type of twinning to be established in some 90% of cases. But merely to ask the parents their opinion is unreliable. Parents tend to be influenced by what they are told in the neonatal period by the midwife or obstetrician who usually erroneously assume that dichorionic twins must be DZ. Any errors in the assignment of the type of twinning will lead to an underestimate of heritability by the twin comparison method.

(2) An estimate of heritability based on the difference between the intraclass correlations of MZ and DZ twins is confounded by any substantial degree of assortative mating of the parents for the character in question. Insofar as any parental resemblance is genetic this will raise the DZ correlation, but not the MZ correlation, and so lower the estimate of heritability below the true value. Assortative mating for intelligence test score in most surveys is high, about 0.5. Adams *et al.* claim that the effect of such assortative mating on the estimates of heritability is likely to be small. This is not correct. For example, on the simple model of purely additive inheritance, with 100% heritability of intelligence test score, and 0.5 genetic parental correlation, the expected genetic DZ correlation is not 0.5 but 0.75. To assume the value of 0.5 would give an h^2 of only 50% and not 100%. Jensen's quoted estimate of an expected DZ correlation of 0.55 seems implausibly low. It is the difficulty of estimating the expected genetic DZ correlation that make twin comparisons an unsatisfactory method of estimating heritability for any character for which there is substantial assortive mating.

(3) Estimates of h^2 from twin correlations based on only 140 pairs have a large sampling error.

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[1] Adams, B., Ghodsian, M. & Richardson, K. *Nature* 263, 314–316 (1976).

ADAMS *et al.* estimate[1] of an upper limit of 0.373 is based on a sample consisting of 41 MZ and 95 DZ twin pairs. This estimate is subject to sampling error, and if one calculates a 95% confidence interval for the population value of the heritability one obtains an interval ranging from about zero to over 0.6. An exact interval is difficult to establish, but using an estimated s.e. of about 0.2, the value of 0.6 can be regarded as a conservative upper limit.

Thus, while the data the authors present are an interesting contribution to this subject and do not seem to support a heritability value as high as 0.8, the assertion of a 'low' upper limit is not really justified.

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[1] Adams, R., Ghodsian, M. & Richardson, K. *Nature* 263, 314–316 (1976).

IT is perhaps surprising that the authors of a study[1] comparing 'mental abilities' in MZ and DZ twins should place such reliance on tests which are not validated, and were administered in a group situation, where contamination effects are difficult to control. If, as is probable, pairs of same-sexed twins sit next to one another in a classroom during the test, it is perhaps quite likely that they will achieve similar results.

An important but neglected study of twins by Claridge *et al.*[2] seems to have avoided at least some of the methodological difficulties which faced the National Children's Bureau study. Claridge's study compared 44 MZ and 51 DZ twin pairs, aged 16–55, resident in Glasgow. Zygosity was established through comparative blood grouping. Among the battery of tests completed by the subjects were the Progressive Matrices and the Mill Hill vocabulary test, well-established and validated measures of mental ability, individually administered.

The intraclass correlations for MZ pairs on the Progessive Matrices was 0.68, and for the DZ pairs it was 0 46. On the Mill Hill vocabulary test, the intraclass correlation in MZ pairs was 0.85, compared with 0.68 in the DZ pairs. For both tests, the intraclass correlation was significantly higher in MZ pairs. Although the authors do not discuss the implications which these results have for models of the inheritance of 'intelligence', it is clear that these results imply that at least some of the variance in mental abilities in this adult Scottish sample is due to inheritance.

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[1] Adams, B , Ghodsian, M & Richardson, K *Nature* 263, 314–316 (1976)

[2] Claridge, G , Canter, S & Hume, W *Personality Differences and Biological Variations A Study of Twins* (Pergamon, Oxford, 1973)

THE report by Adams *et al*[1] of correlations between mental test scores of MZ and DZ twins raises questions about the variance of obtained correlations in twin studies which were not discussed by the authors. Instead, they used their data to suggest the dismissal of previous estimates of heritability; but if the figures are considered in relation to other findings more complex issues emerge. These are the questions of how the various samplings stand as bases for estimating the same population parameter, and of what other features of the testing and sampling (apart from the well known difficulties of mental testing and the identification of twin types) may contribute to the variance of sample correlations

The picture of twin study findings is represented in Table 1, where Adams' figures are compared with corresponding results from an earlier well-founded report[2], and with others from the review by Erlenmeyer-Kimling and Jarvik[3] which gave figures from 34 studies concerned with twins. The Adams figure for MZ twins lies at the lower limit of previous studies, while the figure for DZ twins lies in the upper part of the range Thus that particular sampling must yield one of the smallest, if not the smallest, difference between such correlations in the whole series of studies. Table 2 shows the statistical significance of the differences. While the Newman figures are not exceptional in this respect, the Adams figures clearly are.

Table 1 Correlation coefficients in the mental testing of twins

Source	Twin type (all reared together) MZ *r*	MZ *N*	DZ Same sex *r*	DZ Same sex *N*	DZ Opp. sex *r*	DZ Opp. sex *N*	DZ All *r*	DZ All *N*
Adams *et al.* (non-verbal group test, 1969)	0.762	41	0.604	55	0.487	40	0.594	95
Newman *et al* (group test, 1937)	0.992	50	—	—	—	—	0.621	51
Erlenmeyer-Kimling *et al* (median values of 34 studies, 1963)	0.87	1082 (in 14 studies)	0.53	11 studies	0.53	9 studies	0.53	2052 (in 20 studies)
Erlenmeyer-Kimling *et al.* (1963) upper limit	0 92		0.87		0.63		0.87	
Erlenmeyer-Kimling *et al.* (1963) low limit	0.76		0.45		0.37		0.37	

Table 2 Differences between correlations for MZ and DZ twins

Statistic	Source Adams MZ	Adams DZ	Newman MZ	Newman DZ	Erlenmeyer-Kimling MZ	Erlenmeyer-Kimling DZ
r	0.762	0.594	0.922	0.621	0.87	0.53
Fisher *z* difference	1 000–0.683		1.605–0.727		1.333–0.590	
σ_z difference	0.193		0.205		0.037	
Ratio	1.642		4.283		20.081	
Significance level	Not sig.		0.01		0.01	

In Table 3, figures are compared across the studies for both kinds of twins. For DZ twins none of the differences are statistically significant, even at the 0 05 level, and the samples could be treated as coming from the same population of such twins; but for the MZs the pattern is such that, whereas the Newman and Erlenmeyer-Kimling samples could be treated as bases for the estimation of the same population correlation, it is doubtful whether the Adams sample can be so regarded

This being so, it seems that Adams and his colleagues may have been hasty in offering their findings simply as evidence for a low heritability estimate, for their study is likely to be either a rather extreme sampling or evidence of a change in a population characteristic. Rather than discussing the implications for estimates of heritability it is important therefore to consider the possibility of change in the population correlation figure for MZ twins.

Table 3 Differences between corresponding correlations in different studies

Statistic	Difference Newman–Adams MZ	Newman–Adams DZ	Newman–Erlenmeyer-Kimling MZ	Newman–Erlenmeyer-Kimling DZ	Erlenmeyer-Kimling–Adams MZ	Erlenmeyer-Kimling–Adams DZ
Fisher *z* difference	0 605	0 044	0.272	0.137	0.333	0.093
σ_z difference	0.218	0 178	0 149	0.146	0.165	0.107
Ratio	2 775	0.247	1'825	0 938	2 018	0 869
Significance level	0.01	Not sig.	Not sig	Not sig	0 05	Not sig.

Since available twin studies span a considerable time, during which advice has been given to parents to treat identical twins as individual persons, this may have had the effect of increasing the within-pair variance. More sampling is necessary to test the present position further, as is a thorough analysis of data to test the hypothesis of a trend over time.

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Adams *et al.* reply—None of the above criticisms substantially alters our conclusions, though some underline the caution appropriate in certain places.

Dr Vetta has to be thanked for showing that assumptions made by Jensen in deriving a formula for heritability estimation are without theoretical justification. It follows that the formula ought not to be perpetuated. As regards the implications of this for our own report we should like to make three points: (1) We calculated heritability both with and without Jensen's false assumptions; (2) the calculation itself (we made a point of saying) was the demonstration of an *erroneous* estimation by incorporation of 'treatment effects'; (3) our main conclusion was based on the comparison of raw correlations.

Carter underestimates the generally accepted level of accuracy of zygosity determination in twins by impressionistic methods. Such methods are routinely adopted, especially under difficult conditions such as the geographic dispersal of subjects. Vandenburg[1] has claimed in a review of the approach, that "it has been shown repeatedly that for large-scale twin studies a few questions about the frequency with which close friends or relatives mistake one twin for the other will provide a sufficiently accurate diagnosis within the limits of the accuracy of the variable under study whether it be a mental test score or a physical illness". Cederlof *et al.*[2], found 98% agreement with blood-typing for responses on one of their items on a mailed questionnaire.

Carter claims, however, that merely asking mothers is too unreliable because they may have been confused by neonatal misinformation. This is quite plausible, but we need evidence about the extent of confusion, especially many years after the births. Cohen *et al.*[3] mailed a questionnaire to 35 mothers of twins in their Louisville twin study. They asked them (among other things) whether the twins were identical or fraternal, and twins were subsequently blood-typed. In only two cases did definite misclassification occur through neonatal misinformation, and there were no other misclassifications. Hence their assertion that (apart from the above) "we did not find any mother who seriously believed that her MZ pair was DZ, nor any mother of DZ twins who believed that her twin pair was MZ". Their general conclusion was that "parental perceptions of identical and fraternal twins were extremely different". The correlations for height at 7 yr in our sample (height being a trait of indisputably high heritability) were: MZ=0.926; DZ$_{ss}$= 0.546. We thus do not believe our twin classification was too unreliable though, naturally, caution would not be unwarranted.

Carter illustrates how non-correction for assortative mating could underestimate h^2. The illustration depends, however, on the prior assumptions of high h^2 and high genetic correlation of mates. Both assumptions become less tenable as MZ and DZ correlations approach identity, as is the case in our sample. He does not explain, moreover, why Jensen[4] estimate of pDZ=0.55 "seems implausibly low". The simple fact is that values higher than that applied to previous twin correlations yielded impossible h^2 values >1.00. Both forms of special pleading (high Δrs implying low pDZ; low Δrs implying high pDZ) necessary to preserve $h^2=0.8$ indeed suggests a limitation of the method, but this does not diminish the finding of insignificant Δrs as an empirical contribution. Our sample size was comparable with (if not larger than) previous studies; bigger ones are unlikely to be forthcoming without transgressing very stringent sampling conditions, satisfying which was our study's main strength.

Harvey Goldstein quotes our conclusion, based on comparison of MZ and DZ$_{ss}$ correlations, and then claims that our "heritability estimate of 0.373" is consistent with an upper confidence limit of 0.6. It should be clear from our paper, however, that that estimate is based on MZ and DZ$_t$ correlations, for non-verbal correlations only, calculated purely to illustrate the inflating consequences of introducing 'treatment effects'. It would be quite indiscriminate to transpose that h^2 value and its 'guesstimated' confidence limit to MZ–DZ$_{ss}$ differences which (1) are not significantly different, (2) are quite different as regards verbal and non-verbal measures, and (3) probably themselves contain treatment effects. Our conclusion of "supportive evidence for zero or low heritabilities" seems to us a more circumspect one.

Bagley seems vague about test validity. The test we used has received some validation and a reference was given. It has been administered to two very large nationally representative samples of 11-yr olds so that excellent information is available about its distributional properties and correlations with other measures, teachers' impressions and so on. It should be pointed out that there is no universally validated and standardised intelligence test in this country, which explains the interest in the long-gestating British Intelligence Scale. As well as face-validity, ultimate validation is always by school attainments/teachers' perceptions; the items in our test conformed closely to the half dozen or so types used in conventional intelligence tests[5]. Bagley is quite wrong to uphold the Claridge *et al.* study as a model. For example, vocabulary test is not a usually accepted intelligence test and the Progressive Matrices has been far from satisfactorily validated and is also a group test. Moreover, it is a test for subjects of '11 years plus', whose use on very much older people for the purpose in mind is dubious. It is the problem of testing such a wide age-range of twins (16–55 yr) especially if the ranges for MZ and DZ twins are quite different, that has flawed previous studies and makes ours (of uniform age) unique. Furthermore, the small number of DZ pairs in the Claridge *et al.* study did not allow separation of opposite-sex and same-sex twins so that their (significant) correlation differences could quite easily be explained by the sort of 'treatment effects' which our own study suggests. Finally, the contamination effects of which Bagley speaks, relating to same-sex twins, are as applicable to MZ as to DZ twins, making separation of DZ$_{ss}$ and DZ$_{os}$ correlations all the more desirable.

Francis is wrong to think that we used our data primarily to suggest the dismissal of previous estimates of heritability. It is the doubts about the empirical sufficiency of previous estimates which have suggested their dismissal. Accordingly, it seems quite wrong to treat our findings as co-extensive with previous ones as if they were of equal empirical sufficiency. Again the Newman, Freeman and Holzinger study sample twins of a very wide range of ages and their small numbers of pairs did not allow separation of the DZ$_{os}$ and DZ$_{ss}$ pairs. Similarly, the well known Erlenmeyer-Kimling and Jarvik study includes studies of widely different measures, many with very small samples, wide age ranges, etc. Thus we dispute Francis's inference that our results, from a nationally representative group of the same age, can be explained away by 'extreme sampling'; indeed, speculation of that sort seems a pointless exercise. Of course a changing 'population characteristic', and a 'labile heritability', is a possibility, but debatable only with comparable studies; the studies of Cohen *et al.*[3] and several others, moreover, suggest that identical twins are still treated much more similarly than are fraternal twins.

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Mechanism of stomatal movement

ACCORDING to Bowling's malate-switch hypothesis[1] of stomatal action, the *p*H changes in the guard cell create a gradient of monovalent malate towards either guard cells or epidermal cells causing a diffusion of K$^+$ (bound to monovalent malate) along with the gradient. His theory is based on (1) changes in *p*H of guard cells during opening or closure, (2) balancing of K$^+$ by the amounts of malate and chloride present in epidermal tissue, and (3) no change in the net amounts of K$^+$, Cl$^-$ or malate in epidermal tissue.

Changes in the *p*H of guard cells do occur[2,3], however. Malate and Cl$^-$, together, can balance K$^+$ present in guard cells[4-6]. There is also no net change in K$^+$ of epidermal tissue[7]. The replaceability of K$^+$ by Na$^+$ for stomatal opening demonstrated that a monovalent ion is necessary to increase the turgor pressure of guard cells but not necessarily K$^+$ (refs 8–10).

We cannot reconcile these data on malate with the proposals of Bowling[1]. The epidermal tissues produce appreciable quantities of malate and the changes in malate levels are closely and positively correlated with the stomatal aperture[5,6,11]. Further, the epidermal tissues contain highly active enzymes not only for malate synthesis but also for malate degradation[12,13]. The above data, together with the autoradiographic studies indicate that malate production and metabolism could occur in guard cells[2,11-13]. The general understanding is that guard cells can derive malate through phosphoenolpyruvate carboxylation and may exchange those acid ions for K$^+$. The stimulation of stomatal opening by phosphoenolpyruvate[13] supports this assumption.

In fact, no anion is required for K$^+$ uptake by guard cells since the guard cell can itself release H$^+$ ions[14]. We therefore feel that, in addition to malate, photosynthetic and/or respiratory electron transport-mediated H$^+$/OH$^-$ efflux could cause a change in the *p*H and provide the necessary H$^+$ ions for exchange with K$^+$. We have presented evidence for such light-induced efflux in guard cells[15]. An active uptake of K$^+$ is likely because the stomatal response to even CO$_2$ free air is sensitive to metabolic inhibitors[16]. Abscisic acid also interferes with energy metabolism in guard cells[17,18]. Active K$^+$ transport is, however, not ruled out by Bowling[1].

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BOWLING REPLIES—In developing the malate-switch hypothesis I considered the evidence for a connection between overall malate levels and stomatal aperture quoted by Das, Rao and Raghavendra[1]. I do not think, however, that the evidence is as strong as they imply. For example, Pearson and Milthorpe[2] showed that with a malate concentration of 5 μg mg^{-1} the stomatal aperture ranges from 4 to 24 μm. Allaway[3] qualifies his conclusions by stating that his results were obtained after 3-h treatments in light or dark, and questions whether the malate–aperture correlation exists in other conditions. Willmer and Dittrich[4] found no difference in the rate of incorporation of ^{14}C into malate in the light or the dark by *Commelina diffusa*. They point out that changes in stomatal aperture seem to be much more rapid than do changes in overall levels of organic acids and other metabolic products

I put forward the malate-switch hypothesis after realising that there is a transport dimension as well as a metabolic dimension to stomatal functioning. A hypothesis which assumes that stomata open as a result of synthesis of malate in the guard cells and subsequent exchange of H$^+$ ions with K$^+$ has also to explain how K$^+$ moves to the guard cell from three cells away as it does in *Commelina communis*. An accompanying anion is surely required and on present knowledge this would seem to be malate.

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Scrotal asymmetry

FOR the record, I report that since reading Morgan and Corballis[1] on the subject of scrotal asymmetry and Rodin's dyslexia, I have made regular observations of the asymmetry in one person who is right handed, has not got situs inversus totalis (or even partialis), has never had surgical procedures on the abdomen, and has no neurological, endocrinological or psychological disorder, except a certain impatience with pretentious nonsense. My findings are that if the subject of my investigation had been the model for Rodin's *L'Age d'Airan*, Rodin would have been correct in assigning a lower position to the right testis compared with the left. I am not dyslexic.

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This correspondence is now closed. Ed.

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reviews

Caricature of Darwinism

R. C. Lewontin

The Selfish Gene. By Richard Dawkins. Pp. xi+224. (Oxford University; London, 1976.) £2.95.

POWERFUL theories that offer to explain large parts of the world of appearances carry, immanent within them, their own caricatures. Indeed, their liability to absurd reduction is precisely in proportion to their original generality and power. There are inevitably those who, dazzled by the great insights of others but understanding them only superficially, push these theories far beyond their valid domain of explanation. This has been the fate of the three powerful conceptual systems that have had such an immense impact on the life and thought of the nineteenth and twentieth centuries: Marxism, Freudianism and Darwinism. Marx's historical materialism has been vulgarised by an 'economism' that attempts to explain the last petty detail of social organisation and history by direct reference to 'material conditions'. Carried away by Freud's theories of the unconscious, and especially the transformations of behaviour described as repression, inversion and sublimation, facile 'psychologism' has searched for an explanation of the French Empire in Bonaparte's relationship with his parents.

The caricature of Darwinism which sees the wonderful workings of natural selection in every aspect of the living world and its artefacts, began immediately with the publication of the *Origin of Species.* The enthusiasm that led the early partisans of Darwin to see every difference in the morphology and physiology of organisms as adaptative, also led them into speculation no different from the baroque rationalisation of Paley's *Vestiges of Creation.* Evolutionary theory was rescued from its excesses by Huxley's ideas of allometric growth which enabled us to understand that many morphological features of organisms are simply epiphenomenal consequences of relative changes in different growth fields or in different dimensions, and by Sewall Wright's demonstration that precisely the same selective forces can lead different populations to quite different phenotypic compositions because of random processes operating in a field of multiple stable equilibria. Thus, we no longer feel called on to explain in an adapative mode why the Indian rhinoceros has one horn whereas the African rhinoceros has two.

For more than forty years evolutionary theory has remained free of a naive selectionism, but in recent times there has been a return to the extreme form of the adaptationist program, as evolutionists have rediscovered behaviour. Beginning with the undoubted truth that behaviour must, like morphology and physiology, be subject to the force of natural selection, the new Panglossians end with the old error that *all* describable behaviour must be the direct product of natural selection. The scientific manifestation of this trend can be seen in every issue of say, *The American Naturalist,* which is permeated by the language, if not the formal apparatus, of game theory, and in the development of the school of 'sociobiology', among whose more extraordinary productions is a recent highly praised dissertation explaining *fellatio* and *cunnilingus* among the upper middle classes as an adaptive response to constant resources. The popular manifestation of this new caricature of Darwinism reaches its most extreme form in *The Selfish Gene* by Richard Dawkins.

The Selfish Gene is the result of Dawkins' discovery of vulgar Darwinism. "We are survival machines—robot vehicles blindly programmed to preserve the selfish molecules known as genes. This is a truth which still fills me with astonishment" (preface). I too find Dawkins' description of the relationship between gene and organism rather astonishing. The theme of our passive manipulation by our gene captors is repeated over and over. "They swarm in huge colonies, safe inside gigantic lumbering robots, sealed off from the outside world communicating with it by tortuous indirect routes, *manipulating it by remote control.* They are in you and me; they created us *body and mind*; and their preservation is the ultimate rationale for our existence. They have come a long way, those replicators. Now they go by the name of genes, and we are their survival machines" (p21). "It leaps from body to body down the generations, *manipulating body after body* in its own way and for its own ends, abandoning a succession of mortal bodies before they sink into senility and death" (p36) (my italics throughout).

The form of reasoning that leads Dawkins, or, rather, the evolutionary theorists of whom Dawkins is the enthusiastic reporter, to this view of gene and organism is exemplified in the discussion of crowding and fertility. It is observed that in some animals overcrowding reduces the birth rate. Given this observation, Dawkins then asks "Why does natural selection favour females who reduce their birth rate when the population is overcrowded?". He then contrasts a population selection argument, unfavourably, with a "selfish gene" theory. But both arguments assume without proof that females reduce their birth rate for an adaptative reason, rather than regarding lower fertility as a mechanical concomittant of the altered endocrine function that results from excitement. They also assume that at some time in the evolutionary past the ancestors of these animals did *not* respond to crowding by lower fertility and that genetic variation of just the right sort conveniently arose to allow some animals to do so. Finally, they invent a plausible story to explain why lowered fertility is a 'good thing'. With a little ingenuity, of course, such stories can always be invented, but it is not clear whether we are dealing with science or high-table wit. The realisation that he is engaged in game playing after all comes briefly to Dawkins' consciousness in his discussion of R. L. Trivers's story of reciprocal altruism. "There is no end to the fascinating speculation which the idea of reciprocal altruism engenders when we apply it to our own species. Tempting as it is, I am no better at such speculation than the next man, and I leave the reader to entertain himself" (p202).

Dawkins is not content that his readers should amuse themselves with the clever games already produced by Hamilton, Trivers and Zahavi, but introduces, in his last chapter, his own patented variant. Allowing that the details of human culture are probably not coded in our genes (we are not such robots, after all, it seems), Dawkins suggests that ideas themselves are units of reproduction and natural selection. He asserts, for example, that "The idea of hell-fire is, quite simply, *self-perpetuating,* because of its own deep psychological impact" (p212), although he does not tell us how its "deep psychological impact" provides a mechanism for its self-perpetuation. He shrugs off without analysis the much more plausible and clearly causal hypothesis that hell-fire is not *self*-perpetuating, but is perpetuated by some people because it gives them power over other people. In fact, both Dawkins' suggestion that ideas are replicating selected units and the sociologists' notion that the details of human social activity are coded in our genes and selected for maximum reproductive potential, arise from the same fallacious view of human society, which, in turn, is a reflection of their confusion between materialism and reductionism. Although it is true that human beings are material objects whose brains are the result of a developmental process under the influence of genes, it is not true that their *minds* can be understood when we understand their genes. In like manner, although human society is the product of the sensuous activity of material individuals, human society is not simply the collection of all individuals.

According to Richard Dawkins, "If superior creatures from space ever visit earth, the first question they will ask, in order to assess the level of our civilisation is 'Have they discovered evolution yet?' ". I think it more likely they will want to know whether we know the difference between properties of sets and properties of their members. □

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Compendium of physiology

Introduction to Physiology. Vol. 3. By H. Davson and M. B. Segal. Pp. x+656. (Academic: London; Grune and Stratton: New York, 1976.) £9.80.

THE appearance of the third volume of this series so shortly after the first two (for review see *Nature* **259**, 428; 1976) is a great tribute to the industry as well as the erudition of the authors. It is a pity, even so, that they have been outpaced by inflation and the cost per page has risen from about 13p to 15p.

This third volume is devoted mainly to the homeostatic control of the principal mass-transport systems of the mammal—the circulatory, respiratory, alimentary and urinary systems. There are also sections, in the Bernard tradition, on thermoregulation, the control of plasma electrolytes and blood sugar, and a concluding one on the internal environment of the central nervous system in which the writers are, of course, very much on their home ground. Each of these topics is covered in considerable depth and the bibliography which is appended to each section is copious including virtually all workers who have achieved prominence in their respective fields over the past 20 years as well as many whose contributions are less familiar.

What seems to be emerging is a compendium of contemporary physiology rather than an introduction to the subject. The various sections, although providing detailed and thoughtful surveys of current ideas, suffer somewhat from a lack of contour lines which might help the novitiate to distinguish the salient features in the detailed scenario which is presented. The text is complemented by frequent clear and apposite illustrations, although in one instance, at least, the figure contradicts what is written elsewhere and occasional pictures of apparatus hardly merit the space they occupy. In the text itself there are some confusing and inconsistent statements but these are rare and could well result from hasty proofreading. Also from time to time an idea is tantalisingly floated without being adequately developed.

Now that the half-way stage in its production has been reached, the question must arise as to what type of user is likely to gain most from this multivolume treatise. My own impression is that it is too detailed to serve as a working source book for the average medical student who may, however, find it valuable for occasional consultation. On the other hand, Honours undergraduates in most UK universities would probably be encouraged to read original papers rather than rely on summaries, however well prepared. This leaves postgraduate students who, particularly on taught courses, could find their needs well catered for, and finally there is the harassed teacher who may be called on to give lectures or tutorials outside his own speciality. Persons in the last category should be heartily grateful to Drs Davson and Segal for having so diligently 'researched' such a wide range of important topics and having so skilfully blended and packaged the fruits of their labours.

R. V. Coxon

R. V. Coxon is Reader in Human Physiology at the University of Oxford, UK.

Isotachophoretic analysis

Isotachophoresis: Theory, Instrumentation and Applications. (Journal of Chromatography Library, Vol. 6.) By F. M. Everaerts, J. L. Beckers and T. P. E. M. Verheggen. Pp. xiv+418. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 160; $61 50.

THIS is an important book. Its senior author, Dr F. M. Everaerts, was introduced to isotachophoresis some twelve years ago by his PhD supervisor, the legendary A. J. P. Martin. Since then Everaerts and his group have developed the technique from a laboratory curiosity to arguably the most versatile method available for the characterisation and determination of low to medium molecular weight electrolytes. It is a method which in my view is destined to become one of the standard tools of chemical analysis.

Essentially the book is a full report of the work done by Everaerts' group; as such the treatment of the subject is uneven. The theoretical section does not provide a good introduction to the subject. The long chapter describing a mathematical model of isotachophoresis seems out of place and adds little of practical value. The qualitative aspects of isotachophoresis, however, are adequately described and the chapter on ionic mobilities summarises the variables available for exploitation by the analyst.

The section on instrumentation is by far the best. It provides a clear account of the problem of detection and of presently available solutions to it. The descriptions of the apparatus are complete enough for anyone with sufficient skill to build an analyser for himself.

The applications section is the most disappointing of all. In spite of the wealth of experimental data presented, there are no comparisons made with rival, well established techniques. A careful perusal of the separations depicted in (say) Fig. 12.4 (p 303) and Fig 15.1 and 15.2 (pp 350–351) will convince any analyst of the value of the technique.

In spite of its shortcomings this book ought to be read by all analysts of electrolyte solutions. Scientific instrument manufacturers should also find it of considerable interest, and possibly very profitable.

Frank Hampson

Frank Hampson is a Research Fellow in the School of Molecular Sciences, University of Sussex, UK.

ASA Monograph

Social Anthropology and Law

edited by Ian Hamnett

April/May 1977, x + 234pp., £5.50/$10.75 0.12.322350.4

Here contemporary social anthropologists and academic lawyers present new ideas and provide answers to some important theoretical problems in the anthropology of law. The focal point of enquiry in this volume consists of the relative importance of "norms" or "rules" on the one hand, and of "interest" or "power" on the other, in the settlement procedures of widely varying African societies. This controlling theme involves a close discussion of the role of law in society generally with particular reference to cases in which legal pluralism results in dynamic social, political and economic change.

Studies in Cross-Cultural Psychology Volume 1

edited by Neil Warren

April/May 1977, xvi + 212pp., £8.50/$16.65 0.12.609201.X

This is the first volume in a series which aims to gather together the scattered but increasingly voluminous information on the subject of cross-cultural psychology. The essays demonstrate the peculiar diversity and potential scope of cross-fertilization between anthropology, ethnology and psychology. In principle, cross-cultural psychology might address itself to problems anywhere within the psychological spectrum. In practice, however, certain areas of research lend themselves better to this approach than others, and accordingly the authors have each chosen important growth points, presenting either a report of sustained research program, or a critical review examining and developing conceptual schemes and theories.

Theory and Practice in Interpersonal Attraction

edited by Steve Duck

April/May 1977, x + 438pp., £9.80/$19.25 0.12.222850.2

This book closely examines the nature of interpersonal attraction, what it is, why we are attracted to some people rather than to others, how it is possible to measure the extent of attraction and attractiveness, and how our lives may be affected by the degree of attractiveness or unattractiveness attributed to us. The book is arranged into three sections following three fundamental questions. These are. 1) How do specialist workers explain the phenomena in the area? 2) What sorts of study do they do to investigate the area? 3) How does this area fit in with theoretical work in other areas of the subject.

Proceedings of the Serono Foundation No. 11

Prolactin and Human Reproduction

edited by P.G. Crosignani and C. Robyn

April/May 1977, vii + 306pp., £10.50/$20.50 0.12.198345.5

This book records the proceedings of the eleventh International Symposium of the Serono Foundation, held at Cernobbio in July 1976. The purpose of the symposium was to gather together experts in human prolactin that they may present and discuss recent findings concerning the influence of prolactin on the endocrine control of human reproduction. The result was a comprehensive revision of the relationship between prolactin and human reproduction.

Contents
Assay Methods. Physiology. During fetal life and development. Role in the control of fertility: in animal, in man. **Pathology.** Characterization of hyperprolactinemic amenorrhea. Management of hyperprolactinemic amenorrhea.

Proceedings of the Loch Lomond Symposium, Ross Priory
University of Strathclyde, in September 1975

Studies in Mother—Infant Interaction

H. R. Schaffer

April/May 1977, x + 478pp., £14.80/$29.00 0.12.622560.5

These papers take a lively, modern approach to the topical problem of mother—child interaction, taking care to present the relationship as a dyadic one: the effect of the child's presence on the adult is as considerable as that of the parent on the infant. The approach is marked by the adoption of microanalytic techniques of study, showing how unsuspected patterns can be revealed by close analysis of the extremely complex interactive behaviour which is in existence between mother and child long before linguistic communication develops.

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Carcinological fauna of Japan

Crabs of Japan and the Adjacent Seas By Tune Sakai Volume 1: English text+figs. Pp. i–xxix+772. Volume 2; Plates Pp. 250. Volume 3 Japanese text. Pp. 460. (Kodansha: Tokyo; Antiquariaat Junk Lochem, The Netherlands, 1976.) Dfl.550 the set

DURING the past two decades research on the carcinological fauna of Japan has made rapid progress, resulting in many additions to the species of crabs. The author of this handsomely produced, up-to-date, and definitive monograph has kept abreast with the recent literature and incorporated the results of over forty years of field work and research. Considerable changes in classification and nomenclature have therefore been made—for example, to the family Potamonidae after Bott, 1970, and to the genus *Uca* after Crane, 1975.

About 885 species of Brachyura, referable to 329 genera and 30 families, are dealt with taxonomically, and nearly all are illustrated. Numerous keys are given to the determination of most categories from superfamilies to subspecies Exceptions are, for example, species of *Ebalia,* which require revision; and genera in the Alliance Xanthoida, because Mme Guinot's investigations are still in progress. Well known species, or ones described in the earlier monograph, or in *The Crabs of Sagami Bay* (1965), are not described again.

Throughout, references and synonymies, material examined, habitat with occasional ecological notes, type locality and general distribution, are given. Mention is made of some recently described species not seen by the author. Quite a large percentage are endemic; a few are known only from Japan and some remote locality like Ceylon, or Madagascar. Many are widely distributed throughout the Indo–West Pacific.

Because of the great economic importance of some of the cold water crab-like Anomura of the family Lithodidae, fifteen species (two new to science) are described and illustrated in an Appendix. Bibliographies for the Brachyura and the Lithodidae, and a systematic index, complete the English text. The author's English is always easy to follow, and the Japanese text is apparently intended for the use of students and non-specialists.

The colour plates are excellent About 600 beautiful paintings after living or frozen crabs, specially prepared by the author's wife, are reproduced. and a number of rather striking colour photographs of crabs in their natural habitats are included The book will be useful for specialists working on the fauna of the whole of the Indo-Pacific region.

Isabella Gordon

Isabella Gordon recently retired after many years as Head of the Crustacea Section at the British Museum (Natural History), London, UK

ISIS bibliography

ISIS Cumulative Bibliography. A Bibliography of the History of Science formed from ISIS Critical Bibliographies 1–90, 1913–65. Vol. 3; Subjects Edited by M. Whitrow. Pp 678. (Mansell Information: London, 1976.) £28.

THIS massive work brings one stage nearer completion a major scholarly project reviewed in these pages five years ago (*Nature*, **237**, 115–116) when the initial two volumes were published. The *Isis Cumulative Bibliography* aims to make accessible the rich information on "the history of science and its cultural influences" stored in the critical bibliographies routinely published by the official journal of the History of Science Society. Volumes 1 and 2 recovered and reorganised material under the headings of "personalities" and "institutions". This third volume deals with "subjects". "Periods" will occupy a fourth volume.

The difficulties inherent in this kind of bibliographical synthesis are commensurate with its magnitude. The abbreviations for the journals cited run to seventy-six closely-printed quarto pages The classification scheme needs ten pages of explanation That scheme allows the more than twenty thousand entries to be arranged and cross-indexed under approximately seven thousand categories In an interesting reflection of historical priorities, works on mathematics and the physical sciences occupy one hundred and twenty-three pages to a mere thirty for the biological sciences and twenty-nine for the sciences of man

The present volume allows one quickly to identify the reliable historical literature on subjects ranging from "Adsorption: Chemistry" to "Alchemists: North American: Collective Biography", and "University and Advanced Level Teaching. Mathematics: Russia" to "Writing and Reviewing: Science". Like its predecessors, this volume is an indispensable reference work for all those concerned to understand science as a human enterprise

Arnold Thackray

Arnold Thackray is Chairman of the Department History and Sociology of Science, University of Pennsylvania, and will be a Visiting Fellow at All Souls College, during 1977–78.

Underwater optics

Marine Optics By N. G Jerlov. (Elsevier Oceanography Series, No. 14.) Pp xiii+231. (Elsevier: Amsterdam, Oxford and New York, 1976.) Dfl.77; $29.75.

AT first sight this book seems to be a glossier and larger version of Jerlov's *Optical Oceanography* (Elsevier Oceanography Series, No 5, 1968), indeed the earlier book is still advertised on the dust cover of the present book, which seems a little strange A closer look shows that the number of figures has increased from 83 to 137, tables from 28 to 37, but pages only from 173 to 203. Impressively, the references have increased from 296 to no less than 548, of which nearly 200 are post-1968. This certainly confirms the author's statement that progress in ocean optics is being made at a rapid rate.

In revising the book the author has eliminated some earlier references and included many others, as well as adding so much new work. In particular one should mention the large number of Russian and eastern European references (about 70) in the recent volume, compared with about 30 in the earlier one The author's technique in revising has been mainly to repeat word for word the 1968 book, here and there inserting new paragraphs or tagging on bibliographical information. The extra 30 pages are mostly made up by the extra figures and tables.

It should be stressed there is almost no re-writing of the 1968 text and thus it becomes necessary to justify the production (and purchase) of the new volume purely on the extra information included. The price of about £18 does seem high but the eminence of the author and the assembling of so much extra material probably does justify such expenditure. The new data probably are better shown as figures and tables, but the main advances over the past eight years are more in the nature of additional data built on to old, rather than the formulation of new concepts.

One of the most valuable new sections deals with the use of underwater optics to check the movement of water masses, a valuable tool for the physical oceanographer. As in the 1968 version, however, the author disappoints the biologist reader who is looking for the application of underwater optics to such phenomena as primary production, bioluminescence and animal behaviour

J. H. S. Blaxter

J H S Blaxter is Senior Principal Scientific Officer at the Scottish Marine Biological Association, Dunstaffnage Marine Research Laboratory, Oban, Argyll.

GABA

GABA in Nervous System Function. (Kroc Foundation Series, Vol. 5.) Edited by Eugene Roberts, Thomas N. Chase and Donald B. Tower. Pp. xv+554. (Raven: New York, 1976.) $30.00.

THE identification of γ-aminobutyric acid (GABA) in mammalian brain in 1950 led, after a short lag phase, to a profusion of physiological and biochemical studies. Many of these were described in the only previous volume on GABA and the nervous system, published in 1960. At that time the possible neuronal inhibitory role of GABA, and its importance in epileptic activity, were the focus of attention. A subsequent period of doubt was followed by one of consolidation. An inhibitory transmitter role for GABA in the mammalian cerebellum, cortex and hippocampus is now generally accepted. Experimentally, epileptic convulsions can be induced by drugs which block the synthesis of GABA or impair its inhibitory action. It has yet to be shown, however, that epilepsy in man (except for pyridoxine-related syndromes) arises from a failure in GABA-mediated inhibitory processes.

The present volume arises from a workshop held in California in 1975. The 37 chapters are by 47 contributors (32 from North America, 10 from Japan, 2 from Norway, 2 from West Germany and 1 from Australia). The predominant emphasis is on biochemistry, cytology and cellular physiology. The last 5 chapters discuss the possible involvement of GABA in disease and therapy.

There are indications that we are entering a new growth phase in GABA research. Suggestions that abnormal functioning in GABA systems contributes to the pathogenesis not only of epilepsy, but also of Huntington's chorea, Parkinsonism, schizophrenia, senile dementia, and anorexia nervosa, are providing the stimulus.

The techniques and basic data presented in this volume provides the means. Of outstanding importance is the development of immunocytochemical techniques which, using antibodies prepared from purified enzymes (glutamic acid decarboxylase and GABA transaminase), enable visualisation of cellular structures containing these enzymes. Pioneering reports from the laboratory of Eugene Roberts, describe the light and electron microscopical localisation of these enzymes. These reports provide beautiful confirmation of neurophysiologists' earlier conclusions about which cell types release GABA in the cerebellum and hippocampus. **Brian Meldrum**

Brian Meldrum is Senior Lecturer in the Department of Neurology, Institute of Psychiatry, London, UK.

Polymerisation

Thermodynamics of Polymerization. By H. Sawada. Pp. xiii+403. (Dekker: New York and Basel, 1976.) 130 Swiss francs.

THIS book is an expanded version of the collection of articles written by Dr Sawada for *Reviews of Macromolecular Chemistry* over the period 1969 to 1974. Each chapter has been written as a separate entity so that there is inevitably a certain amount of repetition of both content and references.

The first two chapters are introductory, dealing with the general ideas of ceiling temperature, heats and entropies of polymerisation. These are followed by five chapters covering in succession anionic, cationic, radical, condensation and ring polymerisation. Next comes a chapter on polymerisations, the equilibrium positions of which have been measured, followed by two on copolymerisation. Finally there are two chapters devoted to degradation of polymers and miscellaneous topics such as tacticity and pressure effects.

The reader who comes to the subject afresh may be surprised to discover how much of the book is concerned with kinetics rather than thermodynamics. A more complete description would have been "Kinetic and thermodynamic aspects of processes relevant to polymerisation". Thus under anionic polymerisation we find a discussion of equilibrium between different types of propagating species, under cationic polymerisation an account of the thermochemistry of ionisation processes, and under radical polymerisation substantial sections on the thermodynamics of the initiation, termination and transfer reactions. The kinetic data are sometimes linked with thermodynamically-based parameters such as Hammett σ, and sometimes simply expressed in terms of energies, entropies and volumes of activation. Other times there is only the most tenuous link with thermodynamics, as in the discussion of reactivity ratios in terms of the Alfrey–Price Q,e scheme.

The chief merit of the book is that it brings into a single volume for the first time most of the available information on thermodynamics of polymerisation, and a valuable feature is the Addendum of recent publications.

K. J. Ivin

K. J. Ivin is Professor of Physical Chemistry at Queen's University, Belfast, Northern Ireland.

Wax esters and non-polar lipids

Chemistry and Biochemistry of Natural Waxes. Edited by P. E. Kolattukudy. Pp. xx+464. (Elsevier: Amsterdam, Oxford and New York, 1976.) Dfl129; $49.75.

THIS book is dedicated to Professor A. C. Chibnall who first became interested in waxes fifty years ago and (with S. H. Piper) carried out some classical studies in this field. Significant advances in our understanding of wax chemistry and biochemistry had to await the chromatographic and spectroscopic procedures which have revolutionised and activated all areas of lipid research. The waxes (broadly interpreted) have shared in these developments, and for any who have overlooked them the record is fully documented in this book. Chemists and biochemists interested in lipid methodology, lipid structure, and lipid biosynthesis, and biologists of many kinds will want to consult this book and to have frequent access to it.

The term "wax" is not here confined to wax esters but includes a wide variety of non-polar lipids: hydrocarbons, ketones and aldehydes, primary and secondary alcohols, ethers, alkanediols, acids, wax esters of several kinds, and terpenoids. In general these may be saturated or unsaturated and straight chain or branched chain. The editor has chosen, however, to organise the chapters on the basis of the type of organism from which the wax is derived, the ten contributions being: introduction to natural waxes (P. E. Kolattukudy); mammalian waxes (D. T. Downing); marine waxes (J. R. Sargent, R. F. Lee and J. C. Nevenzel); bird waxes (J. Jacob); and their biochemistry (P. E. Kolattukudy); insect waxes (L. L. Jackson and G. J. Blomquist); plant waxes (A. P Tulloch); and their biochemistry (P. E. Kolattukudy, R. Croteau and J. S. Buckner); algal and fungal waxes (J. D. Weete); and bacterial waxes (P. W. Albro). Dr Kolattukudy is to be congratulated on securing the service of such authoritative contributors. In addition to his own writing the editor has—more or less successfully—prevented undue overlap of material between the various chapters.

At appropriate places in the text, structure and composition, structural determination and analytical procedures (chromatography and several kinds of spectroscopy), biosynthesis and catabolism, function, and taxonomy are discussed. **F. D. Gunstone**

F. D. Gunstone is Professor of Chemistry at the University of St Andrews, UK.

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obituary

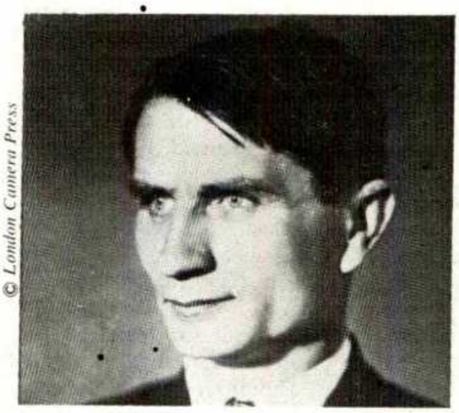
T. D. Lysenko

IT IS a paradox that Trofim Lysenko, who died at the age of 78 on November 20, 1976, was for 30 years the best known spokesman for communist science both inside and outside Russia. For he was not a member of the Communist Party, he was hardly a scientist, and of course he never left Russia. It was his part in the fluctuating relations of successive heads of the Soviet Government with science and with agriculture in the Soviet Union that aroused this world-wide interest.

What first brought Lysenko to notice was his claim in 1930, based on his work at a Ukrainian plant breeding station, that pretreatment of grain would enable Russian farmers to sow winter wheat in spring and thus to reap an increased harvest. This claim, made in the year of Stalin's 'great break', was soon linked with wider generalisations.

It was said that such treatments would change heredity, displacing the slow methods of orthodox plant breeding. Hence they would revolutionise both the practices of agriculture and the theories of biology. For sceptical observers it was not clear whether these claims were based on scientific experiment or philosophic expectation. But it was believed that they were the result of collaboration with a Marxist mentor, Professor I. I. Prezent, who for long remained in the background. Nevertheless, first scientific and then political journals in Russia began to discuss them. Eventually the newspaper *Pravda* and the whole Soviet press was seen to be accepting the evidence and propagating the arguments of Lysenko. The demand arose for a public discussion of the relative merits of Western idealistic bourgeois genetics and the new practical materialistic and Marxist science.

At this point other branches of the Government stepped in. An International Congress on Genetics which was being arranged in Moscow for 1937 by N. I. Vavilov, the President of the Lenin Academy of Agricultural Sciences, was ordered to be postponed. In its place a debate was arranged before a purely Russian and largely agricultural audience. Here the geneticists were allowed to defend themselves and there was a serious argument. But by this time Stalin's purges were in full swing and when the great man himself appeared on the platform and intervened to exclaim "Bravo, Comrade Lysenko" it was obvious how the argument would be settled.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Vavilov had been removed from some of his offices before the meeting. After it was over the leading geneticists, one by one, disappeared. Those who were members of the Party were shot. Vavilov himself was not arrested until 1940. He was then sentenced to death as a British spy (having been a frequent visitor to Britain). He was reprieved but later died in prison. Lysenko took over his chief posts and these he continued to hold for twenty-five years.

News of what had happened leaked out of Russia only after the war. By 1946 critics in Britain and the United States were beginning to make serious charges. Lysenko and the Soviet Government were accused of collusion in scientific fraud and political murder. Lysenko's second active phase then began. He denounced the critics abroad, and any who might support them at home, as enemies of the people.

To make it clear what this meant, a second debate was now arranged. The battle lines were laid down in advance. On the Marxist and Leninist principles of struggle and partisanship, two biologies were seen to be implacably opposed. On one side was Lysenko's own science, honest, creative and revolutionary, marxist and materialist, proletarian and patriotic. On the other side were the false and futile works of the Mendelist–Morganist Weismannists who were hardly human and almost entirely foreign.

The meeting took place in August 1948. Again it was at a time of crisis, the battle over Berlin. A few surviving supporters of genetics were allowed to speak, heckled and ridiculed from the floor. Lysenko's followers filled the hall. They also filled the pages of the proceedings with long citations from Marxist authorities. What their statements amounted to in a positive sense was simple but comprehensive. It was the assertion that the inheritance of acquired characters must be the basis of all plant and animal improvement. From this immense generalisation only man was excluded, eugenics and medical genetics having been abolished in the 1930s. In a negative sense there was another conclusion which could also be put in a nutshell. As Lysenko said: "No efforts of the advocates of the chromosome theory of heredity . . . to lend their theory a materialistic appearance can change the character of the theory which is essentially idealistic" (*Applause*). The obstinate material character of the chromosomes and heredity, which had been unknown to Marx, was thus shown never to have existed.

The climax of the meeting came when Lysenko announced that his report had been examined and as was to be expected, approved, by the Central Committee of the Party (*Stormy Applause, Ovation, All Rise*). After Stalin's death Lysenko added the footnote that his patron had most diligently edited the report before approving it. Well he might, for the 631 pages of the Proceedings translated into many languages were ordered to be circulated in well-bound editions at home and abroad.

At the end of the meeting three professors of genetics admitted their errors and promised to follow Lysenko's guidance in future. Those whose honesty and courage forbade submission were degraded and dispersed. By a series of decrees all teaching and research in genetics, agricultural or medical, was suppressed. Books were destroyed, type broken up and libraries purged throughout the Soviet Union. There followed a period of idolisation of Lysenko. He was now a Hero of Socialist Labour with nine Orders of Lenin. His statue appeared in public places and the State Chorus sang his praises.

Lysenko's career, so far, had been sustained by a stream of projects, like his first proposal, to parallel the work of collectivisation in revolutionising Soviet agriculture. Vernalisation of wheat was followed by inherited vernalisation. Hybridisation within varieties was followed by pollination without hybridisation and hybridisation without pollination. Summer potatoes in the North were followed by spring wheat in Siberia; by a *tour de force* wheat became rye and by love-marriages extraordinary fruit hybrids became fertile, while under the master's hand a hazel sprouted from a hornbeam.

During Stalin's lifetime the succession of claims was too rapid to disprove and too dangerous to contradict. But when he died critics raised their heads. And when Khrushchev, three years later, announced a thaw, they dared to open their mouths. The fact that none of Lysenko's claims had ever been vindicated might now, it seemed, prove to be his undoing. But matters of state are not to be so easily disentangled. As can happen even in a free world the industries of communication and education were now implicated with the establishment in a general deception. They were also infiltrated by the secret police. What was worse, it turned out that the successor, Khrushchev himself, liked Lysenko; not as Stalin did, for his theory but for his earthy language and proverbial wisdom.

So it was that Lysenko enjoyed one last spell of power. The virgin lands of Kazakhstan and the novel possibilities of cattle breeding offered him new fields to conquer. The two men embarked on these ventures in partnership and their failure seems to have contributed to the downfall of both. For Zhores Medvedev's exposure of this failure in *Samizdat* led to his confinement in a psychiatric clinic. But it had been known to the Praesidium before it deposed Khrushchev on October 14, 1964. The customary newspaper campaign followed. But now, for the first time, it was turned *against* Lysenko. He was dismissed as head of the Academy's Institute of Genetics on February 1, 1965. And the Institute itself (not for the first time) was dissolved.

News of these developments was released abroad. But the Russian public were only by rumour allowed to perceive that a great pillar of the state had somehow been withdrawn; and to feel that two systems of belief, abandoning the struggle to the death, were to be allowed to shake down together. When summer arrived, sure enough, the Academy in Moscow was allowed to celebrate the centenary of Mendel's paper and to dispatch to Brno in Czechoslovakia seventy geneticists, the largest of all delegations, to lay a wreath on the Abbot's monument.

Thus everything, it seemed, could now be amicably and democratically arranged. Lysenko, for his part, could remain a member of three academies. He could keep his titles and pension, his following of university professors, his journal regularly published and his Experiment Farm in the Lenin Hills. And the grateful geneticists were free to pick up what they could, first in medical, then in animal, and ultimately in plant genetics, from the ruins that Lysenko had left them. Meanwhile the Government could forget that it had ever interfered in these academic matters.

Outside Russia, however, the damage that had been done to the relations between scientists and communism could never be repaired. The view of communism as meaning the liberation of science, held in the 1920s had been turned inside out. With this painful reservation, inside Russia, it is a hopeful sign that the restored geneticists are proposing to hold in Moscow in August 1977 the International Congress on Genetics so tragically abandoned forty years ago. The history, the theory, and the philosophy of genetics are not among the 35 headings proposed for discussion. But, no doubt, the names of the men whom Lysenko destroyed will be discreetly remembered.

One more question should be asked: was Lysenko a charlatan? It is an important question because fraud is always, and in all countries, bound to collect in the wide fringes of science. He was obviously ill-educated, quite shallow, very cunning and a little deranged. As Vavilov put it, privately, he was "an angry man" of a kind we all know. And he found his anger brought him great rewards. On the other hand, his followers were far from angry. They had calculated the rewards that a corrupt system had to offer them.

It remains to say that the world of science owes a debt to three bodies of men in connection with Lysenko. There were those like Vavilov who died in fulfilling their duty. There are those like Andrei Sakharov and Zhores Medvedev who resisted and survived. And there are those devoted scholars outside Russia, notably David Joravsky and Loren Graham, who have enabled us now to understand so much of what happened. *C. D. Darlington*

S. B. Pessôa

The death of Professor Samuel Barnsley Pessôa, removed the grand master of parasitology from Latin America, a region where the subject has flourished vigorously throughout this century. His predecessors in Brazil included H. de Beaupaire Aragão, Carlos Chagas, H. da Rocha Lima and Oswaldo Cruz. Pessôa himself founded a school of workers who have penetrated into all corners of South and Middle America. The training of the first generation stemmed from their close association with German, French and Italian scientists either directly in the New World or during their own visits to Europe in pre-world war days. Pessôa's monumental book, *Parasitologia Médica*, has gone through many editions and is in constant use in Portuguese and Spanish speaking countries.

Pessôa was born in São Paulo on June 4, 1898, the son of Leonel Pessôa and Anna Barnsley, who was of English descent. He spent the greater part of his life in São Paulo, and died there on September 3, 1976.

After qualifying in medicine, he entered in 1925 the General Prophylactic Service of the State of São Paulo as an experimental officer attached to the Institute of Hygiene. His work took him far afield and he quickly became acquainted with the severe sanitary problems of the rural areas as well as those of the city. The distress and poverty of so much of the population transformed him into an ardent fighter for the abolition of the system, which accepted this situation with equanimity, and his ideas became increasingly revolutionary.

His abilities were quickly recognised both in the academic and in administrative fields: he was appointed to a Chair in the Faculty of Medicine of the University of São Paulo in 1931 and was later made Director General of Health. In the latter role he entirely re-organised the service by starting large scale campaigns against endemic diseases and initiating research projects. He took an active part in the latter and made many original contributions notably in muco-cutaneous leishmaniasis, trachoma and schistosomiasis. His observations on espundia and on the transmission of this form of leishmaniasis were fundamental. He studied the epidemiology of Chagas' disease and disentangled some of the problems of the cardiac complications and other phenomena of 'mega'.

Pessôa's reputation was so great that he was appointed to numerous chairs throughout Brazil and even in his last years, he was still commuting between different universities from one end (e.g. Goiás) of Brazil to the other (e.g. Santa Catarina). Pessôa never hesitated to express his views and he was often threatened with prison or dismissal. In the last 15 or 20 years of his life, he became entranced by the parasitic protozoa of snakes and lizards, working both at the Institute of Butantan and in the field on these parasites, which had always attracted Brazilian scientists; he disclosed many new species and methods of transmission by leeches and other invertebrates.

Pessôa was a colourful character, always welcome in international gatherings, where his astute and original mind was much appreciated. But above all, he was revered by his past pupils who could count both on his loyalty and protection during trials and persecutions, and invaluable advice regarding their scientific problems.

P. C. C. Garnham

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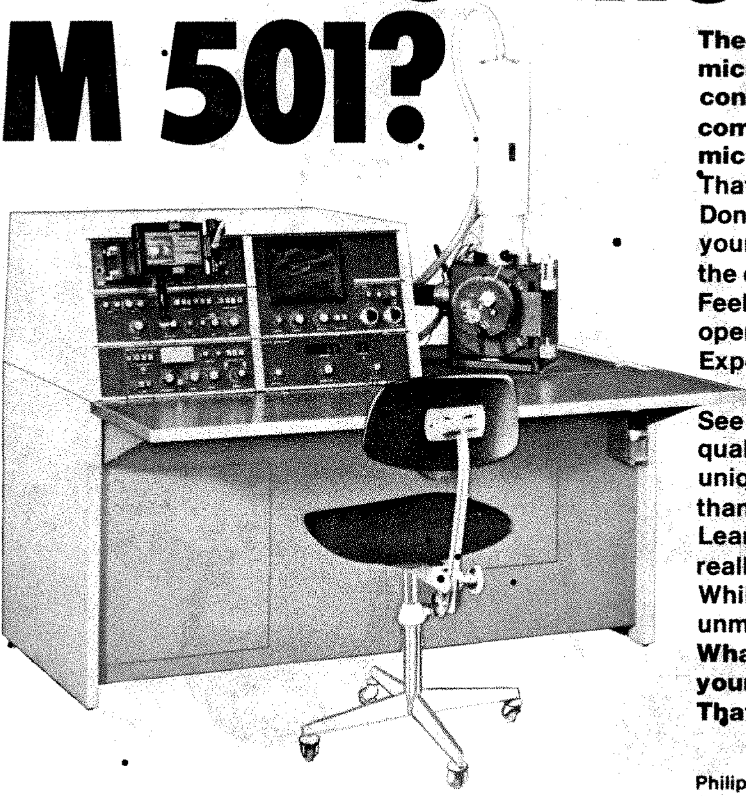

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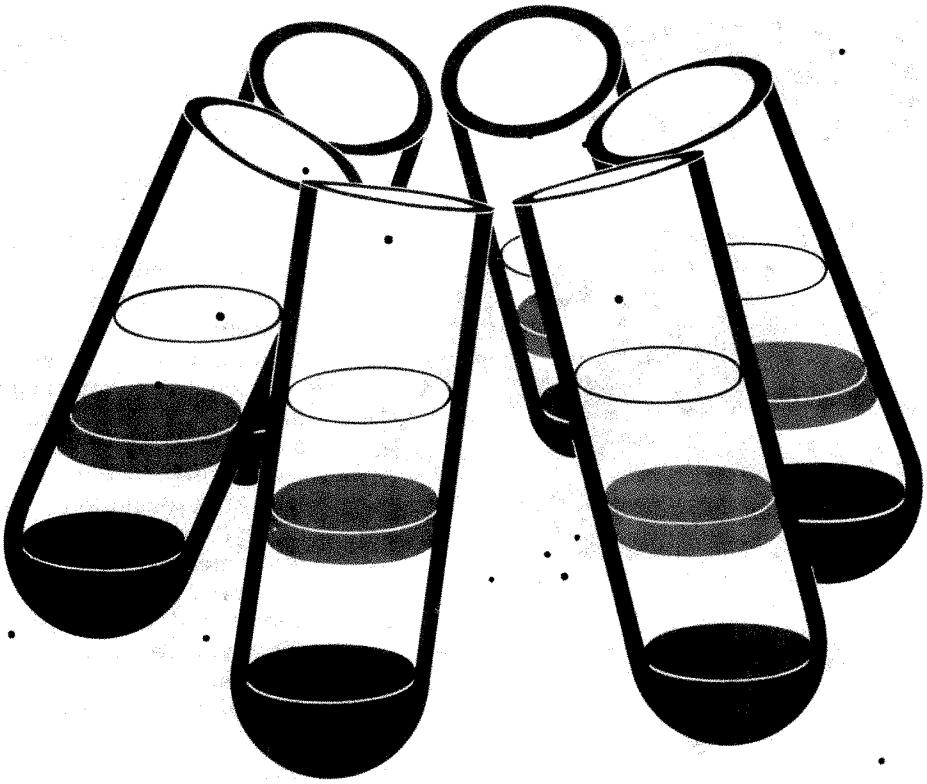

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nature

24 March 1977

For the city and for the world

PROFESSOR Daniel Bell of Harvard and sabbatically at the London School of Economics was lecturing last week at the United States Embassy in London on social institutions in post-industrial society. It was Daniel Bell, of course, who all but invented the term 'post-industrial society' and who has written extensively on the subject of a world in which knowledge is the major resource. He contends that the scale of many of our institutions (in tune, perhaps, with earlier days in which the major resource was land or machinery) is not necessarily in very good register with the sort of demands that will be made in a post-industrial society. An organisation that is co-extensive with a nation or a state may be too large for some purposes, too small for others.

In Professor Bell's *The Coming of Post-Industrial Society* (Heinemann, 1974) it was the scientists and the somewhat quaintly labelled 'research men' who would become the dominant figures where the landowners, the military and, more recently, the businessmen had once been. It follows, then, that the scale of organisations which sponsor, stimulate, monitor or control science is as much in question as in the scale of other social and economic institutions. Is there such a thing as a right size for administering science? This question has been raised recently in Scotland, as Martin Goldman remarked in last week's *Nature* (page 208), but it is not simply one of being ineffective if below some supposed critical mass; on the contrary it may be that the United States, Britain, Scotland, even the Greater London Council are too big for some purposes.

At this small end of the spectrum the local problem with a scientific content has frequently been sadly neglected. Polytechnics do what they can within the heavy teaching load, but universities rarely have much sense of springing from the local community, the academic staff are unlikely to be of local origin and the administration is likely to be more worried by the changing scene in the capital city than in the local town hall. As a result, peculiarly local questions of health, environment, resources and stimulation of enterprise are frequently passed over; besides, scientists are rewarded more by fame than fortune, and you don't get many people receiving wide acclaim for solving a problem at the local quarry or sewage works. There seems to be a whole range of scientific questions on this small scale needing small institutions to initiate them.

But, some will argue, if we are weak at the small scale, we are at least capable of operating, when needed, at the supranational level. Look at CERN IPOD, ESA, ILL; all institutions which work quite well on an international basis. Certainly scientists, when the financial pressure is on, find little difficulty in sinking national differences. This is hardly surprising since scientific knowledge, of necessity, transcends national frontiers. But what is surprising is that this great international spirit has still made precious little impact at the science policy level. One example would be in the field of medical research. Every developed country must be seen to be funding a coherent programme to fight cancer just as it must have its own airline. The health problems of the developing world, arguably much more pressing than those of the developed world, receive a few per cent of our attention and yet we know full well that developing countries are unlikely to have the resources to support adequate research themselves.

There have, of course, been tentative efforts towards regional science policies and talk by individuals of something resembling a global science policy, but as yet the power and pride of nations has not allowed much progress along a direction which is going to cut across a lot of autonomies and cosy relationships. Maybe pressure in this direction will eventually come from the financial problems of supporting extensive national scientific programmes. One would hope, however, that pressure might come from scientists themselves for international decision-making and international programmes over a very wide range of disciplines.

Daniel Bell's challenge is this: to ensure, as knowledge becomes the major resource, that it is sought in the areas where there is greatest need and is distributed fairly and rapidly. We would do well to ponder whether our present institutions are best organised for this task. □

A fair exchange?

Philip Hanson assesses the balance in the East–West transfer of technology

THE technological relations between the West and the Soviet bloc are curious. The United States and the Soviet Union spend huge sums on military R&D, primarily to maintain their abilities to blow one another to pieces. Yet at the same time Western businessmen are eagerly selling know-how and high-technology products to the USSR and Eastern Europe; and there is also a certain commercial flow of technology—of more modest dimensions—from East to West.

These transactions, of course, are in principle restricted by strategic embargoes to non-military purposes; yet the technologies transferred quite often originate from military R&D. In any case the civilian–military distinction is somewhat artificial: in the long run any new technology that makes the civilian economy more productive makes it easier to enlarge the resources allocated to the military. And even if there were no military considerations, economic competition between communist and capitalist countries is part of their general competition for power and influence, and so much technical assistance among competitors—even at a price—is at first sight rather odd. Altogether it is not surprising that East–West technology transfer is politically controversial.

The recent growth of Soviet–US trade, in particular, has prompted economists to look more closely than before at East–West technological relations. They have focused, naturally enough on commercial transactions. Obviously there are many ways in which knowledge about new products and processes travels from West to East without any commercial exchange, but these are not discussed much because most of them are not amenable to control by governments. The screening of Western technical literature by Soviet specialists, for example, and the gleaning of information from visits and conferences are beyond the influence of all but the most provocative action by governments.

East–West trade, however, provides a number of channels of technology transfer: notably, the sale of products embodying new technology; turnkey projects; licencing; know-how sales; and technical assistance and training. Some of these transactions simply provide cost-saving machinery and other products (for example wide-diameter pipe for gas pipelines) without transmitting the know-how to make the product in question. Other transactions, however, such as licence deals with technical assistance, or joint design work, may transfer such capabilities.

An interesting example is the Pullman-Swindell contribution to the huge foundry complex at the Soviet Kama River lorry works. Over a period of three years a total of more than 150 Soviet engineers worked with Pullman-Swindell on the detailed design and equipment ordering. The knowledge they acquired must have been considerable. How far it would enable the Russians to repeat the exercise for themselves probably depends on the extent to which these particular engineers can be kept together as a design team.

Most Western governments subsidise export credit as they compete against one another to promote exports and they thus ease the terms on which technology is transferred. Is this really in the interests of the West as a whole? Would it be wiser not to encourage such transfers, or even to restrict them? What is or is not wise in this context is largely a matter of political judgment: in other words, nobody really knows. The economics of East–West technology transfer, however, are more susceptible to measurement, and a good deal of measurement has accordingly been going on. The aim is to quantify the economic costs and benefits to Western and Soviet-bloc countries of transactions by which technology is transferred.

The starting point is the well established fact that in most fields Soviet and East European civilian production lags technically well behind that of the West even though these countries maintain large R&D establishments and are strong in many areas of basic research. Heavy military priorities, semi-isolation from Western technology and the lack of competitive pressures and incentives, however, have resulted in a generally poor performance in the creation and diffusion of new technologies. They therefore stand to gain a good deal from the acquisition of Western know-how.

Clearly Western firms also gain from selling this know-how. A recent survey of 168 US firms selling to the USSR found—in contrast to what some economists had predicted—that about three-quarters of them considered their activities to be profitable. The position of West European firms, who have had more experience of dealing with the Russians, is probably more favourable.

Novosti

West–East: Fiat's USSR showroom

The transfer that is going on, whether by means of product or of know-how sales, is predominantly of proven technology. In electronics, for example, Western firms quite often sell their latest products or the know-how to manufacture their less advanced products, but they seldom transfer the know-how to manufacture their latest products—let alone those currently under development. There is also, in the case of the Soviet Union, evidence that absorption and utilisation of imported technology is slow by Western standards. As a result, there is so far no very obvious narrowing of East–West technology gaps as a result of transfer.

Western firms, in other words, have not gone into Eastern markets in order to establish Eastern competitors for themselves. It is rather odd that anyone should ever have expected them to. Since they could not set up wholly- or majority-owned subsidiaries in any Comecon country, they have mostly been careful to hand over technology in measured doses: in products, in licence deals subject to market restrictions, and so on. More than a hundred companies, mostly big multinationals, have signed technological cooperation agreements with the Soviet State Committee for Science and Technology, but exchanges of information under many of these agreements have been minimal.

Production benefit

There is a good deal more, however, to the economics of East–West technology transfer than the question of its commercial profitability. Much of the debate in the West concerns the impact of imported Western technology on Soviet production, and this raises some quite different questions. To begin with, there is the question whether imports of Western technology are really of more than marginal importance to the Soviet economy. The USSR has traditionally been highly self-sufficient. Only a very small percentage of its consumption and investment needs are met by imports. Less than half of these imports, moreover, have been from the West, and of this, capital goods—the main vehicle of international technology transfer—have generally comprised about a third. This means that in 1976, for example, imported Western machinery was almost certainly not more than 6% by value of total machinery installed. (The large differences between Soviet domestic prices and the prices of imports make precise comparison impossible.)

At first sight, this suggests that imported Western technology cannot have more than a very minor effect on Soviet output. If the growth of production depends on the growth of the labour force, the growth of the capital stock and technical change, then the technical change embodied in a very tiny part of the increment in the capital stock must surely make only a tiny contribution to that growth.

This need not follow, however, if the technology embodied in the imported machinery has significant indirect, as well as direct, effects. The direct effect of the imported technology is the extra output attributable to it, over and above what would have been produced with the same resources of capital and labour with the technologies already in use inside the country. But this same technology will also have indirect effects insofar as it is successfully diffused (for example by building Soviet machinery to the same design) and insofar as it enables improved producer goods (such as compound fertilisers) to be made which themselves raise productivity in other lines of production.

East–West: exporting machine tools

NOVOSTI

Soviet machinery and know-how imports have been strongly concentrated in certain fields: the main recipients have been the chemical industry, the motor industry, computerisation, shipping, and oil and gas pipeline transport. The planners have identified areas where there was a considerable technology lag: in other words, areas where the direct and indirect impact of technology would be considerable. The production of fertilisers and pesticides is an example: the backwardness of both the Soviet chemical industry and Soviet agriculture promised high returns to hard-currency spending on imported technology.

Some recent estimates suggest that the returns have indeed been high. One tentative estimate for the mineral fertiliser industry is that by 1975 the technology embodied in about $2,000 million worth of installed Western equipment was yielding, directly and indirectly, some $4,000 million worth of extra agricultural output per annum, over and above what the same resources would have produced in the absence of the imported technology. Part of the gain came from the diffusion of Western phosphoric acid and compound fertiliser technology to Soviet built plants.

A global estimate has been derived from an econometric model of the Soviet economy. The conclusion in this case was that, of an overall increase in Soviet industrial output of 32.1% be-between 1968 and 1973, 2.5% could be attributed to an acceleration of the import of Western machinery over that period. The acceleration was such that the stock of installed Western machinery was about a fifth higher in 1973 than it would have been if the rate of growth of imports had remained unchanged. Like the estimate for the mineral fertiliser industry, this entails a very high rate of return.

On the other side of the scale, the contribution of East–West technology transfer to Western GNP is very small indeed. The Soviet Union has probably made slightly over 200 licence sales to Western firms in recent years, mostly unaccompanied by machinery, and often requiring development and adaptation in the West. And the employment associated with Western capital-goods sales to the Soviet bloc is probably less than 0.5% of total employment in most OECD countries.

According to the conventional wisdom, Western governments should not worry unduly about all this because the Soviet economic system is relatively poor at using and diffusing new technology, and this fundamental lack of technological dynamism will keep the level of Soviet civilian technology generally below that of the West, even if the import of Western technology and know-how continues to grow strongly.

One way street

This may well be so. But if the two estimates just described are anywhere near correct they put this conventional wisdom in a somewhat changed perspective. They suggest that, while the Soviet economic system may be slower than Western economies in using and diffusing new technologies, it nonetheless does get a fair mileage out of imported technology. Where there is a particularly large backlog to be exploited, the impact on production can be dramatic.

This means that those who see East–West technology transfer as a 'one-way street', in which the benefits go mainly from West to East, have a serious case. It may be true that imported Western technology is not enabling the USSR to catch up and overtake the West in areas of advanced technology, but it is probably making a significant contribution to Soviet productivity levels, and thus reducing the pressure on Soviet policymakers to divert resources from military to civilian purposes.

Similarly, the firms selling the technology are no doubt doing so on commercially acceptable terms. On the other hand they are generally competing with one another to extract extra revenue from products and processes whose development costs have already been written off; they are therefore unlikely to charge prices that would come close to reflecting either the cost to the Soviet-bloc purchaser of replicating the development work for himself or his gains in output from using the technology.

The balance of economic advantage, then, in East–West trade in machinery and know-how seems to be strongly in the Soviet bloc's favour. From a conventional Western point of view this is not a welcome conclusion. Further evidence might possibly alter it, or it might be made more palatable by clear indications that the growth of East–West trade was linked with political advantage for the West. If not, a rather awkward question arises: is there anything much that Western governments can do about it? ☐

USA

After the carrot, the stick

Colin Norman reports from Washington on the moves to introduce legislation to control recombinant DNA research in the United States

JUST two years ago, at the now famous Asilomar Conference, a group of scientists was discussing a set of proposed guidelines for experiments involving the powerful new recombinant DNA genetic engineering technology Joshua, Lederberg, a Nobel Prizewinner from Stanford who had been sounding ominous warnings about threats to free scientific inquiry, gloomily told his colleagues that there was "a graver likelihood of (these proposals) crystallising into legislation than some of us would like to think" That likelihood is now a virtual certainty.

Last week, the health subcommittee of the House of Representatives held three days of intense public hearings to examine a number of bills, including one proposed by the subcommittee chairman Paul Rogers, which seek to regulate all recombinant DNA experiments in the United States. Next week, the House subcommittee on Science, Research and Technology will begin its own set of hearings. On April 6, the Senate health subcommittee, chaired by Senator Edward Kennedy, will hold a hearing to discuss various legislative proposals already introduced into the Senate, and Kennedy will probably subsequently offer a bill of his own. And legislation which will soon be proposed by the Carter Administration is now taking shape (see below)

Thus, what Lederberg correctly perceived as an inexorable march toward federal legislation is now entering the home stretch It will take several weeks, perhaps months, for a final bill to emerge from Congress, however, and the debate is likely to turn on a relatively small number of issues.

First, it should be noted that Congress is unlikely to try to rewrite the guidelines issued last year by the National Institutes of Health (NIH). Though it is possible that some legislators will offer proposals to ban the research entirely—Representative Richard Ottinger said at last week's hearings, for example, that he is considering offering a bill declaring a moratorium and calling for an international meeting to develop a better understanding of the hazards—it is unlikely that such a move would succeed.

The most probable outcome is that Congress will direct the Secretary of Health, Education and Welfare (HEW) to develop regulations based on the NIH guidelines Both the Rogers bill and the Administration's draft bill take that approach, and Kennedy said in a statement last week that "we must assure sufficient and meaningful involvement of laymen and the general public in the formulation of regulations by the Secretary", thus indicating that he is likely to support that approach as well.

A second point to note is that the final regulations will apply equally to research supported by public and private funds, and that the legislation will establish a mechanism by which the controls can be enforced. Those two elements are missing from the NIH guidelines, and the need to extend and enforce the guidelines is largely responsible for the momentum now behind the development of federal legislation

The coming Congressional debate is thus likely to centre on four areas:

- **The extent to which the federal regulations will override state and local controls.** Many scientists who were once opposed to federal legislation now support it because several state and local governments, led by the City of Cambridge, are developing their own controls on recombinant DNA experiments, thus raising the possibility that a patchwork of regulations of varying strictness will be adopted throughout the United States Most scientists are consequently hoping that federal regulations will specifically prohibit local governments from setting their own controls on the research.

The Administration's draft bill would

A committee of government officials, representing 16 federal agencies, last week urged the Carter Administration to draft legislation to control all recombinant DNA research in the United States, and the committee sketched out the chief elements which it believes should be included in such a bill. As soon as the committee's report landed on his desk, the Secretary of Health, Education and Welfare (HEW), Joseph A. Califano, announced that his department would promptly transcribe the recommendations into formal legislation, noting that "such a measure is necessary not just to safeguard the public but also to assure the continuation of basic research in this vital scientific area".

The bill is expected to be ready early in April, when it will be submitted to Congress with an endorsement from the White House. It is likely to be particularly influential as Congress grapples during the next few weeks with the complex problem of how recombinant DNA research should be regulated.

The inte-agency committee, chaired by Donald Fredrickson, Director of the National Institutes of Health (NIH), began last November to seek ways to extend NIH's own recombinant DNA guidelines to cover experiments supported by other government agencies and by private industry. Though other agencies have since adopted the NIH guidelines, the committee decided that new legislation is needed to ensure compliance by everybody conducting recombinant DNA experiments in the USA

The committee consequently recommended that the Administration's bill should contain the following provisions. The Secretary of HEW should issue regulations based on the NIH guidelines, "with such clarifications and modifications as the Secretary determines to be necessary" All recombinant DNA experiments should be conducted at facilities licenced by HEW, the licence being issued only when the Secretary is satisfied that the facility will be operated in accordance with the regulations. Researchers would have to register their experiments with HEW. The committee suggests, however, that the Secretary could exempt from licencing and registration requirements "categories of activities which he determines pose no unreasonable risk to health or the environment"

To ensure that the regulations are being followed, HEW would have the authority to inspect facilities, conduct environmental and health monitoring, and require that reports and records be kept. The Secretary would have power to halt experiments if necessary, and violations of the regulations could incur loss of a facility's licence. In the event of injury to people or the environment, actions and damages would fall under state and local laws.

The committee suggests that all reports submitted to HEW by experimenters and officials in research facilities should be made available to the public on request, except for information likely to cause loss of proprietary rights Before information is released, researchers would be able to identify material which they believe should be kept confidential, but the final decision on disclosure should rest with the Secretary of HEW.

In what may turn out to be one of its most controversial recommendations, the committee suggests that federal legislation should specifically pre-empt all state and local regulations regarding recombinant DNA research The only exception would be if a state passes a law imposing requirements identical to those contained in the federal legislation, the Secretary of HEW could enter into an agreement with the state to carry out the inspection, monitoring and enforcement responsibilities. In other words, such a clause would rule out the setting of state and local controls of varying stringency.

Finally, the committee recommends that the legislation should contain provisions protecting the rights of employees who blow the whistle on their superiors, and it also suggests that the legislation should lapse after five years unless further action is taken by the Congress indeed pre-empt local moves, though it would allow the Secretary of HEW to enter into agreements with state governments under which local agencies would enforce the federal regulations. The Rogers bill, however, would allow the Secretary of HEW to approve state regulations which are at least as strict as the federal controls, and Kennedy said last week that "we must carefully assess the wisdom of totally pre-empting state and local laws regulating the recombinant DNA research and applications given our concern to assure the involvement of the general public in these issues".

The crux of the issue is how the federal government can ensure the adoption of uniform regulations without throttling public debate and without cutting off legitimate community interest in what universities are up to.

- **The nature of registration and licencing schemes.** It is likely that the final version of the legislation will establish a mechanism through which researchers or research facilities would be licenced and research projects would be registered with the federal government. There is a significant difference in approach between the Administration's draft bill and the Rogers bill, however, and it is not clear which approach will prevail.

The Administration's draft bill, in short, would require that facilities housing recombinant DNA experiments be licenced by HEW and that individual research projects would simply have to be registered with HEW. The Rogers bill, on the other hand, would require researchers themselves to obtain licences from HEW before embarking on any recombinant DNA project, and the licence application would specify the nature of the project and the safeguards to be employed. The difference between the two approaches represents a very significant difference in the focus of control and the complexity of the licencing process. Kennedy has so far made no statement on that aspect of the legislation.

- **The extent to which proprietary information can be protected from public disclosure.** There is a clash of interests between the need on the one hand to provide full public information on recombinant DNA experiments and the need on the other hand to protect proprietary information from public disclosure. That dichotomy is sure to generate a good deal of Congressional debate. The Administration's draft bill and the Rogers bill deal with public disclosure in slightly different ways, though both leave to the Secretary of HEW the ultimate decision on what information should be made public. The Rogers bill essentially requires the Secretary of HEW to publish in the *Federal Register* details of research projects for which he has issued licences, though he would simply exempt from disclosure proprietary information. The bill under consideration by the Administration, however, would require the Secretary of HEW to make information public only on request and after the researcher involved is given a chance to object if he believes that disclosure would jeopardise commercial interests.

- **The nature of sanctions if violations are discovered.** It is likely that the legislation which will eventually emerge from Congress will empower HEW to inspect facilities, and require reports, health monitoring and so on. The legislation is likely to give the Secretary of HEW the power to revoke licences if violations of the regulations are discovered, and he would also be able to halt projects deemed to present an imminent hazard. As for the question of liability, the Administration's draft bill would leave that up to state and local laws, while the Rogers bill would allow imposition of a $1,000-per-day fine for each violation.

Those areas are likely to be the chief focus of discussion in the next few weeks as Congress goes through the unprecedented process of setting federal regulations on an area of basic science. One thing is already clear from the public debate so far, however. As NIH Director Donald Fredrickson put it during last week's hearing: "biomedical research is entering a new era in its relationship to society. It is passing from an extended period of relative privacy and autonomy to an engagement with new ethical, legal, and social imperatives under concerned public scrutiny". ☐

NIH grants: OK, but . . .

After a two-year study of the system used by the National Institutes of Health (NIH) to parcel out more than $1,400 million a year in research grants, an internal NIH committee has concluded that the system is working well, but would benefit from a few changes.

NIH now handles about 15,000 grant applications a year, and funds less than a third of them. They are reviewed first by a study group consisting of a number of scientists working in related fields, which rates the proposal according to its scientific merit and which takes into account such considerations as the track record of the applicant. The proposal, together with the study group's rating, is then passed along to the advisory council of the Institute to which the application has been referred. The council goes into such matters as whether the proposal fits in with the priorities of the Institute, and it recommends whether or not the grant proposal should be funded. The final decision is made by NIH officials. Clearly, the most influential stage of the review is the initial evaluation of the study group.

At present, study groups meet behind closed doors and their recommendations are exempt from general public disclosure. The committee has recommended that the groups continue to deliberate in private, but it has suggested that the groups' conclusions and recommendations routinely be communicated to grant applicants as soon as the Institute's Advisory Committee has completed its review.

On the question of confidentiality, the committee has also expressed concern about the fact that grant proposals, once they are funded, must be made available to the public, on request, under the Freedom of Information Act. The committee urges passage of legislation protecting grant proposals from public disclosure.

As for the support of unorthodox research, some concern has been expressed that the peer review system favours traditional, conservative research at the expense of creative, unorthodox research. The committee therefore suggests that applicants identify innovative aspects of their research proposals. It also suggests that the study groups prepare a statement pointing out the innovative aspects of a grant application.

Finally, the committee recommends that a formal appeals system be established in NIH through which applicants can appeal the assignment of their proposals. It suggests that an ombudsman be appointed to adjudicate disputes over the handling of grant proposals, and that a Permanent Grants Peer Review Appeals Board be established under the chairmanship of the ombudsman.

The committee's recommendations are now being considered by the NIH Director, Donald Fredrickson. He is expected to endorse most of them.

Colin Norman

EUROPE

First meeting

THE problem of disclosure of information emerged last week as the most immediate obstacle to progress in formulating European guidelines for recombinant DNA research when representatives of bodies in some 15 European countries concerned with its regulation met in Strasbourg under the European Science Foundation's wing.

The meeting, the first of the newly established European Committee on Recombinant DNA, was from all accounts very useful and friendly but far too short to allow adequate discussion of a heavy agenda. The disclosure issue concerned the details which need to be revealed about particular projects in order that guidelines might be formulated and containment procedures applied without the applicant surrendering scientific or commercial advantage.

The simple origin of the problem is the recommendations of the committee's predecessor. This concluded last year that the recommendations and code of practice of the British Williams report should be adopted as guidelines for recombinant DNA research in Europe. The difficulties arise because those recommendations are general rather than specific, emphasise physical more than biological containment, and operate in a country where agreed standards of confidence obtain and certain patent laws operate.

Since only a body of case law can fill in the grey areas created by the lack of specificity, the argument goes, access to full details of particular cases becomes necessary for everyone using the guidelines, particularly where biological containment allows a drop in the level of physical containment. The trouble is, in Britain disclosure of such detail apparently means forfeiting the right to apply for a patent, and the hard-to-win cooperation of such companies as ICI with Britain's Genetic Manipulation Advisory Group (GMAG) must be on the understanding that revelations to it will not constitute disclosure.

Whether that means that without full disclosure the Williams guidelines could not operate fairly is unclear, but it is apparent that Britain's patent laws, interpreted in this way, could create a problem nationally and certainly internationally. The position is different in the United States, where application for a patent can be filed up to 12 months following disclosure.

In Strasbourg the French and Swedish representatives urged full disclosure, and the representative of Britain's GMAG was not in a position to agree. That means that answers are needed on the precise meaning of Britain's patent laws as they relate to recombinant DNA research before the next meeting expected in September.

No one seems to doubt that there should be a full exchange of information for any European guidelines to work properly. But the potential economic rewards of the work (the bad experience of penicillin is not lost in Britain) are also recognised, and if industry is to be incorporated in the whole scheme without resort to legislation, it may be at the price of not having full disclosure. In that case, the idea of using paradigmatic cases will gather support.

Chris Sherwell

BRITAIN

Sea-bed disposal: safe after all?

An optimistic assessment of the radiological consequences of disposing of solid high-level radioactive waste on the ocean floor is published this week. Chris Sherwell reports

IMAGINE it is the year 2010. The world's nuclear power programme, consisting entirely of light water reactors, had an installed electrical generating capacity ten years earlier of 2,500 GW. The high-level waste produced by that programme has since been cooling in ponds at a reprocessing site, and has now been glassified and put into stainless steel cylinders with 'waste oxide incorporation'. The cylinders—all 72,000 of them—are about to be dumped on the floor of the North Atlantic, in one operation. What will happen over the next 1,000 million years?

An attempt to answer that question is contained in a report out this week from the National Radiological Protection Board (NRPB)* and prepared for British Nuclear Fuels Ltd (BNFL). The subject is especially controversial at the moment in Britain where, apart from the decisions being sought on the choice of the next generation reactor and on the commitment to the fast breeder, the matters of oxide fuel reprocessing and of high-level waste disposal remain unresolved issues.

Just last week, for example, the UK Atomic Energy Authority (UKAEA) attached great importance to the words of Dr Frank Feates, head of Harwell's Environmental Safety Group, at a public meeting in Scotland. The search now being conducted was not for dumping grounds in any particular area, he said; it was to see if unfissured granite, no matter where it was, was a safe structure for high-level waste disposal.

The idea of underground burial, which is being investigated in other countries too and focuses on salt and clay as well as granite, is only one of three options. The others are burial under the ocean floor and placement on the ocean floor, and it is the consequences of the latter alone which the NRPB study examines, even though the London Convention of 1972, which came into force in 1975, expressly forbids such disposal—a point the study mentions in an appendix.

The report endeavours to model the ways in which material deposited on the ocean floor will re-enter man's immediate environment, especially via his food. The principal route of return it uses is via dispersion in the deep ocean, physical transport to the productive surface layers, incorporation in marine food chains and the consumption of contaminated seafood, but it also considers radiation doses arising from contamination of beach sediments.

Although the report emphasises that only broad conclusions should be drawn, it states that "no overriding reason connected with radiological protection considerations has been identified which would preclude the disposal of suitably conditioned high-level waste on the ocean floor". Other points:

- All the vitrified waste may be expected to be dissolved in about 3,500 years. The heat generated by the wastes would not give rise to "any significant vertical transport mechanism". The amount of activity removed from the water by adsorption onto sediments will not be the major fraction of the total amount released, and will not significantly deplete the quantity of activity in the water, for 10^5–10^6 years.
- Assessments, for each of the many nuclides involved, of their changing concentrations over time in surface and deeper waters, and of how these are take up by plankton, molluscs, crustacea, fish and eventually man, show the relative importance of different routes. The ratio of intake to the maximum permissible annual intake (I/MPAI) attains its highest value for consumption of deep-ocean fish or plankton (neither of which routes currently exist) at times of either 50–100 years or 500–2,000 years. The ratio is 10^{-2}; for surface fish, the order is 10^{-4}; other ratios are below 10^{-5}.
- The largest annual collective dose is due to consumption of surface fish (the only intake route actually established), at about 4×10^4 man rems at 50 years from ^{137}Cs and ^{90}Sr taken together. Collective doses at longer times will be of the order of 10^2–10^3 man rems per year. The figures would be larger if plankton was established as a major direct food source.
- Predictions of nuclide levels in the sea water do not exceed the level of the nuclides known to be present in sea-water naturally or through fall-out.

The study emphasises that different conclusions might be reached if the waste was separable into short- and long-lived fractions, if a different composition (or even monolithic) glass was available, if better containment materials than steel were used, or if additional barriers, including even the sediment itself, were employed.

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*P. D. Grimwood and G. A. M. Webb, *Assessment of the Radiological Protection Aspects of Disposal of High Level Waste on the Ocean Floor.* **(NRPB-R48, HMSO, 50p).**

● Conservation is one of the strongest refrains in the UK Department of Energy's (DEN) efforts to produce an orchestrated energy policy. Straightforward measures like fuel pricing policy and speed restrictions have been buttressed by various schemes like the Save It campaign, the Industrial Energy Thrift Scheme, the Energy Audit Scheme and the Energy Survey Scheme; a recent report on Energy for Industry from Cambridge Information and Research Services has emphasised the importance of conservation in any future strategy; a DEN report on energy elasticities published last week speaks of the savings to the balance of payments through cutbacks in consumption; ministers—last week it was Peter Shore, the Environment Secretary—regularly stress the importance of conservation. And on the international front Britain is now taking part in two of four R&D programmes on conservation through the Paris-based International Energy Agency.

To some it is obvious, to others exotic, but one of the more ambitious schemes mentioned in Britain to encourage conservation has now received an official judgment. It comes from a working party of a special group set up under the aegis of the DEN's Advisory Council on Research and Development (ACORD). The group is the Combined Heat and Power Group and the working party's focus was its domestic application—that is, district heating. The group's main report, which is unlikely to be out this year, will cover industrial applications.

No one has doubted that changing the operation of a conventional power station to produce vast quantities of hot water but less electricity can allow substantial energy conservation. The problem is whether it can be done economically, and last week's report, *District heating combined with electricity generation in the United Kingdom* (HMSO, £3.75), gives a carefully quantified response.

The difficulties in making calculations are enormous. One reason is that it would take some 15 years to develop a heat load suitable for connection to a combined heat and power (CHP) plant. Moreover, a new and expensive system of lines would be necessary to distribute heat to consumers; and even then a great deal depends both on the demand from them (what will their standard of living be?) and their distance from the power station (what planning policies will apply?). The future cost and availability of alternative fuels and the development of alternative technologies such as heat pumps is also important. So, although the savings in energy are clear and the engineering involved is established, the assumptions that are made when using standard discounted cash flow techniques are critical.

For all this, the working party concludes that with present fuel prices and fuel availability, and with the test discount rate used by the Treasury of 10%, there is no economic incentive to introduce CHP and district heating save for limited high density areas. CHP could be more economic with a reduced discount rate, however, or if fuel prices rose, or both. Fuel saving would amount to 20 mtce compared with the 1972 fuel consumption pattern if CHP plant supplied 25% of the population.

At the turn of the century, on the other hand, when oil and gas will be scarce and expensive, CHP and district heating would be economically attractive compared with direct electric heating or with substitute natural gas produced from coal. About 25 GW less capacity of nuclear power stations would be required compared with direct electric heating if CHP plant supplied 25% of the population, and fuel savings would be somewhere between 7 and 30 mtce.

But if a significant amount of district heating network is to be installed by the first decade of the next century, the working party says, it is necessary to start now, and that means selecting a pilot city for conversion; strong government initiatives are essential if CHP is to develop significantly. Last week, however, Dr John Cunningham, the minister with responsibility for conservation, and Dr Walter Marshall, chief scientist at the DEN, were both at pains to emphasise the political sensitivity of embarking on such a course without proper consideration of the people affected.

● The trouble with the report out this week from the Wellcome Trust covering the 1974–76 period is that there is no indication of the price levels to which its figures refer. Thus the Trust says it allocated over £8.5 million to research in human and animal medicine, which is some 91% higher than the nearly £4.5 million it allocated in the previous 1972–74 period. Inflation was such that £1.35 million of the increase went to meet salary increases for people the trust supports. And it expects an income of £5½ million in 1976–77. But are these and other figures at 1974 or 1976 prices? Answer: the figures are at prices at the time referred to.

The present strength of Wellcome, which is the largest endowed charitable trust supporting medical research in the United Kingdom, is, according to the report, "the result of the expansion of the international pharmaceutical company, the Wellcome Foundation Limited." For the 1976–77 allocation it has had to make a choice between deliberate support of special objectives and *ad hoc* selection from the applications the trustees received. It has opted for a more selective policy for 1977 focusing on such relatively neglected areas as ophthalmic medicine, the vascular system of the human brain in relation to disease, the metabolic effects of infection and the pathology of trauma. There is no increase in funds for projects submitted for *ad hoc* consideration.

● Participants in this weekend's conference in Aberdeen on the changing sea-bird populations of the North Atlantic will gather just five weeks after the latest serious incident involving oil pollution. To the concern of many, at least 700 oiled birds, and possibly twice that number, came ashore near the sites of Yorkshire's cliff-nesting seabird colonies. Faced with the argument that official maps showed no vulnerable bird concentration in the area for that time of year, the Royal Society for the Protection of Birds had to organise its own aerial survey to find and size the oil slick responsible; and the RSPCA actually destroyed some live oiled birds instead of sending them to a new and costly rehabilitation centre. The adequacy of existing maps and the criteria for bird rehabilitation remain important issues to ornithologists. With the subject of pollution on the conference agenda, there could be a bit of a flap in Aberdeen.

Chris Sherwell

Quality control

Vera Rich writes on the reorganisation of higher degrees in the Soviet Union

THE Soviet media are at present conducting a major campaign eulogising Soviet achievements, as part of the build-up to the forthcoming 60th anniversary of the October Revolution. The achievements of Soviet science feature conspicuously in this campaign. In one field, however, there is little cause for rejoicing, and the press notices strike a critical note—the training of the next generation of scientists and the awarding of higher degrees.

In the Soviet Union, the degrees of Candidate (roughly equivalent to a PhD) and Doctor of Science are awarded not by the universities, but by a special body, the Higher Qualifications Commission (*Vysshaya Attestatsionnaya Kommissiya*, VAK) of the Ministry of Higher Education. At the beginning of 1976, new regulations for higher degrees came into force, setting new, higher standards and reorganising the structure of VAK. In its new form, VAK consists of a 'Plenum', a 'Presidium' and a 'College', with 34 'councils of experts' dealing with the principal lines of research, and 500 'specialist councils' for assessing degree theses, 150 of these councils being entitled to deal with doctorate theses.

Under the new regulations, a higher standard is demanded, both in the theses themselves and also in the official opponents against whom the theses must be defended. Postulants for a higher degree have, in addition, to fulfil certain non-academic requirements; according to Professor V. G. Kirillov-Ugryumov, the Chairman of VAK, they are expected to "combine a profound professional knowledge with a mastery of Marxist–Leninist theory and with the convictions of an active builder of communist society."

In spite of this emphasis on *partiinost'* (Party-mindedness), the changes were primarily intended to improve the standards of scientific manpower. The first year's work of the reorganised VAK has, however, failed to effect this improvement, and the overall picture suggests incompetence and inadequacy.

According to *Pravda*, a number of the degree councils have not been observing the proper procedure for the defence of theses and have been deliberately lowering their standards for reasons ranging from "bonds of friendship" to the "exploitation of official status" by the postulant. Twenty-one degree councils have been suspended as a result of such infringements. Nevertheless, during the year, almost 20% of doctoral theses were rejected by VAK.

Theses appear to be of a low standard because more than half the postulants come from industry, having carried out their research while working in jobs which may not necessarily be closely related to their academic work. This is a somewhat delicate matter to broach in the media; Soviet policy constantly urges a closer contact between research and industry, and even so eminent a body as the Siberian Branch of the Academy of Sciences of the USSR was recently censured for slackness in establishing and maintaining such contacts.

However, Moscow radio recently hinted at some of the difficulties facing the young graduate who goes straight into production and attempts, while working, to obtain a higher degree. Those who embark on such a course, suggested the commentator, should be "surrounded by care and attention". In plants where this had been done, working scientists had obtained degrees not only at the Candidate level but even at the Doctor level. Directors who failed to cherish in this way their ambitious young scientists "display shortsightedness and lack of foresight". The difficulties of combining research with a job, even a job in one's chosen field, were considerable, and accordingly, while in no way overtly criticising official policy, the commentator urged that special attention should be paid to ensuring that more young graduates gain their research experience in the most authoritative establishments.

The current Five-Year plan is aimed at increasing efficiency and quality in all sectors of production, including the higher education 'industry'. The reorganisation of VAK was but one aspect of this drive towards quality. The first results of the new system show that it is not easy to achieve higher standards simply by legislation. □

More give, more take

DR Mikhail Shtern was unexpectedly released last week from the labour camp where he was serving an eight-year sentence for allegedly accepting bribes. The official TASS statement on his release maintained that the sentence was "correct", but claimed that acting on considerations of socialist humanity, and in view of Dr Shtern's poor state of health, the Supreme Court of the Ukrainian SSR had decided to reduce the sentence to two years and nine-months—the term already served.

After his release, Dr Shtern told foreign reporters that he was "absolutely not guilty of anything", and that the accusations against him were made in reprisal for his sons' application to go to Israel. Dr Shtern's release followed a worldwide protest campaign which involved fifty Nobel Prize winners. A special tribunal to examine the Shtern case was scheduled to open in Amsterdam this week.

Dr Shtern's release, however, does not appear to augur a relaxation of pressure on the refusniks. In Moscow, Yosif Begun, an electrical engineer, is in prison awaiting charges of "parasitism", that is, being without visible means of support—a charge which has not been brought against any refusnik for some time. On 15 March, the day after Shtern's release, Anatolii Shcharanskii, the mathematician who has acted as press agent for the Azbel' Seminar and is closely associated with the Moscow 'Helsinki monitoring group', was arrested while actually in the company of two Western journalists who were interviewing him about the Shtern case. Shcharanskii's family has been told that he may be accused of "crimes against the State", an accusation which could lead to a show trial.

Jewish activists in Moscow see the arrest of Shcharanskii as part of a growing campaign of repression against the refusniks. Although there have been recent attacks in the media on other dissidents too, the campaign against the Jews who simply wish to leave the Soviet Union is far more aggressive than that against the Russians, Ukrainians, Lithuanians and others who also urge change.

The anti-refusnik campaign has been particularly directed against the scientists and academics. Searches of unprecedented severity have taken place at the homes of several of them, resulting in the destruction of their books and the confiscation of all papers. At the time of his arrest Shcharanskii, together with Professor Aleksandr Lerner, a cyberneticist, and Vladimir Slepak, an engineer, were attempting to bring a libel suit against the government newspaper *Izvestiya*, which had accused them of spying for the CIA.

Vera Rich

COMECON

● The first meeting of the Heads of the Academies of Science of the Comecon countries took place last month in Moscow. All nine Comecon members were represented, and also Vietnam. Yugoslavia, however, which has a special relationship with Comecon in a number of fields, including science and technology, was not represented. The meeting consisted of an exchange of views on the development of cooperation in solving key problems of "the building of socialism and communism", and increasing the efficiency of scientific research and the implementation of research results in production—this last being the most pressing problem for R&D in all Comecon countries.

Examples of cooperation to date were discussed, including joint programmes for space research, earth sciences, physics, chemistry, and biology. Special attention was given to the role of the scientists in détente and the duty of scientists "recognising their responsibility before history" to use their influence in support of non-proliferation and disarmament. The scientists of Comecon, it was stated, are ready to unite their efforts with those of the scientists of all other countries to work on "major problems of concern to all mankind", such as energy, raw materials, environment, the rational use of ocean resources, and epidemiology. Suiting his tone to the occasion, Mr Brezhnev, in welcoming the delegates to a Kremlin reception, proposed a law of composition for scientific cooperation between socialist countries—individual efforts, he asserted, were not added but multiplied together.

● The recent Romanian earthquake has raised certain doubts concerning earthquake prediction. This is an important topic of research in the Soviet Union, and the countries of Eastern Europe have watched developments in the field closely. In 1976, a Soviet team produced a seismic map of the Balkans predicting probable epicentres for the next 100 years. It would seem, though, that the foretelling of natural disasters is a mixed blessing.

The suggestion of American seismologists that an earthquake of the same magnitude with its epicentre in Romania might be repeated within a few weeks was denounced on Bulgarian radio as "an act . . . beyond all bounds of the admissible and even of the inadmissible in inter-State and inter-human relations", being designed to "create a panic among ordinary people". Whether the Romanians coping stoically with the aftermath of the disaster were alarmed by the American predictions, or even had the opportunity to hear them, is a moot point; but in Yugoslavia considerable alarm was created in the Krusevac region by radio hams who predicted that an earthquake of even greater intensity was imminent.

The only predictions that have not been pilloried by the East European media have been the non-alarmist ones—that of the Institute of Geophysics of the Bulgarian Acadamy of Sciences, which announced comfortingly that the periodicity of an earthquake of this magnitude was of the order of 50 years, or that of Professor Bruce A. Bolt of the University of California who, after visiting the site of the Romanian earthquake, pronounced that present scientific methods do not allow one to say whether such a phenomenon might occur in the near future.

According to Dr Negmatullyaev, director of the Soviet Institute of Seismic-Resistant Construction and Seismology, who is at present working on earthquake prediction in Tadzhistan, current Soviet methods, using boreholes and gas-analysis, allow earthquakes to be forecast with the greatest degree of accuracy. Reactions to the Romanian disaster would suggest, however, that until prediction can be backed up with prevention, the publication of predictions may present a problem in public relations and media management.

Among the victims of the Romanian earthquake were Professor Florin Ciorascu, Corresponding-Member of the Romanian Academy of Sciences; Academician Paul Petrescu, a physicist; Professor Carli Marcu, a pharmacologist; and Professor Marin Lupu, Rector of the Bucharest Academy of Economic Sciences. Major scientific installations damaged included the National Physics Centre in Bucharest, the Faculties of Chemistry and Medicine at the university and an 'atomic centre'.

● Since the May 1974 visit to Britain of Academician Vladimir A. Kirillin, Chairman of the State Committee for Science and Technology of the USSR, when he declared that industrial fast-breeder generators would not be a practical possibility before the mid-1980s, Western commentators have from time to time suggested that long-range Comecon planning in nuclear power generation is committed to fast-breeders. Such speculations were recently revived at a press conference which Kirillin gave to the heads of foreign missions in Moscow. On this occasion, after referring to the successful operation of the pilot fast-breeder reactor at Shevchenko, Kirillin stated that such reactors would be in industrial use within the next ten years.

Such estimates, however, seem over-optimistic. The recent meeting of the Comecon Permanent Commission for the Peaceful Uses of Atomic Energy in Riga, which reviewed progress to date and approved future plans, paid special attention to the designing of 1,000 MW water-moderated water-cooled reactors. Since this is above the capacity of any reactor scheduled for production in the plans to 1980, any major changeover to fast breeders cannot be expected in the present planning period.

One of the major topics of discussion at the meeting of the Commission was specialisation by the member countries in the production of isotopes, instruments, and equipment for power generation. Yugoslavia, although not a member of Comecon, has a working arrangement by which she participates in a number of Comecon projects, including the Interatomenergi programme for the construction of nuclear power station equipment. Several engineering plants in Yugoslavia are already participating in this construction, including the Litostroj combine in Ljubljana. Litostroj has designed a special circular crane, to operate in complete isolation inside a reactor, to charge it with nuclear fuel and remove the waste products. The equipment is intended for power stations in the USSR; a recent report on Yugoslavia's energy resources and demands makes no mention of nuclear power, but stresses the importance of home-produced coal as the basis of the electrical generating industry.

Vera Rich

IN BRIEF

German nuclear problems

The domestic and international aspects of Germany's activity in the nuclear field have both come in for further dispute recently. Following the visit of the US deputy Secretary of State to Germany a fortnight ago, and the visit to Washington last week of Germany's Foreign Minister, the German position on its controversial nuclear deal with Brazil apparently remains one of determination to fulfil its obligations, in spite of US objections.

Domestic developments have proved less restrained. Violent demonstrations occurred at the weekend outside a nuclear plant in Lower Saxony; and news of a third court case on the construction of a nuclear plant, this time in northern Bavaria, followed the decision last week of an administrative court in Baden-Württemberg to withdraw the construction permit for a plant on grounds relating to safety. A Schleswig-Holstein court blocked construction of a plant at Brokdorf last month.

Press statement

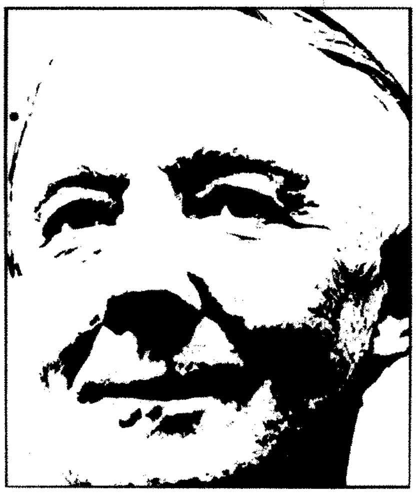
The long-awaited official announcement finally came late last week: Frank Press, Professor of Earth Sciences at MIT, has been named Director of the White House Office of Science and Technology Policy (OSTP) and science adviser to President Carter. The appointment must be confirmed by the Senate, but no problems are anticipated there. Press's appointment has been a certainty for the past month, but the official announcement was delayed by a lengthy series of security and other background checks which President Carter has insisted be made on top officials in his Administration. Press has been working at the White House, however, attending meetings of Carter's advisers and helping select candidates for other top research jobs in the government.

NSF budget change

In an unusual move, the House Committee on Science and Technology has restored some of the cuts in the budget request for the National Science Foundation (NSF) made by the subcommittee on Science, Research and Technology. The subcommittee had voted to hold NSF's budget increase for fiscal year 1978 to about 7% compared with the 11% boost requested by the Administration. The full committee has restored about half the cuts made by the sub-committee in NSF's basic research and Research Applied to National Needs (RANN) programmes, additions which would give NSF a 9% increase.

According to staff aides on the committee, the full committee took the unusual step of overturning the subcommittee's recommendations because of recent projections that the inflation rate will be higher than the subcommittee had assumed.

Fishing line



KENNETH MELLANBY

THE most impressive success story in conservation is the recovery of the population of the Antarctic fur seal, described by Nigel Bonner of the British Antarctic Survey in a recent *NERC News Journal*. Before 1791, when the first sealing expedition went to South Georgia, these animals existed in millions. By 1830 they were so scarce that the fur sealing industry stopped. However, they survived in small numbers, perhaps less than a hundred animals, and were officially protected by the British administration of South Georgia in 1907. Most zoologists thought this action was too late and that the species was doomed. But now estimates for 1976–77 suggest that there are over 300,000 and that the population has increased annually by at least 17% for the past 20 years. The seals should soon reach their former level of abundance.

One reason suggested for this phenomenally rapid increase is the ample food supply. Fur seals feed mainly on krill, the shrimp *Euphausia*. This is also the food of the whales which have been so severely reduced in numbers. Successful whale conservation could leave less food for seals. However it seems more likely that man will soon compete for krill, and this could affect both whales and seals. It is therefore interesting to assess the value of this new human food. Would the gain in human nutrition be sufficient to make it worth putting these marine mammals at risk?

The world catch of marine fish for 1974 was estimated at 60 million tonnes. Biologists believe that it would be possible to harvest almost as great a biomass of krill—at least 50 million tonnes per annum. This would seem at first to add greatly to the food resources available for the growing world population. The ocean's contribution to man's food is surprisingly small. Much of the fish is used to feed livestock and pets. When fed to farm animals, the wastage is considerable, and broiler fowls and pigs yield much less protein and energy than the fish supplied to their diet. Even when eaten by man, much of the carcass is wasted. Probably less than 30 million tonnes of actual fish flesh is eaten by humans each year. Even with the krill, the actual consumption would be unlikely to exceed 50 million tonnes. In terms of energy (calories or megajoules) the total world fish catch would satisfy some 30 million people—less than the annual increase in global population. Even the krill would be consumed by a few month's increase.

Of course, this calculation is, or should be, absurd. Fish is a rich source of high class protein, and the annual catch could supply the protein needs of a tenth of the world's population, and the krill could meet the demands of a further two hundred million individuals. Unfortunately, it is unlikely that it would do so. It is rare for fish to be eaten except by those whose diet already contains sufficient protein. Those who live on staples low in protein, including cassava, plantains and yams, seldom obtain much fish, and it is unlikely that they would benefit from krill. Fish would almost certainly go to increase the luxury eating of man and beast in the richer countries.

We should therefore be cautious before encouraging the exploitation of krill. We see that many of our traditional fisheries are overexploited, and may be destroyed unless internationally conserved. We are very ignorant about the interdependence of krill, seals, whales and man. As the oceans, in spite of overexploitation, are making such a trivial contribution to feeding the world's increasing population, we must realise that most of the food needed must be grown on dry land.

correspondence

Science with public appeal

SIR,—I think your criticism of the BBC's programme, *The Key to the Universe* (3 February, page 393) was unfair to say the least. Nigel Calder did not make any "fundamental mistakes", especially in underestimating the serious mindedness of the audience. Indeed his refreshing attitude encourages the public to take a greater interest in science—we would soon fall asleep (at least I would) if the programme was presented in the way you implied in the article. These two-hour programmes are occasional. they are not the same as *Horizon* and they add a new look to the debate.

I think you are making the fundamental error by assuming that "the uninitiated 99.9% of the audience can only have been thoroughly confused . . . ". I did not find the order in which the programme was presented at all confusing. Your leader writer should watch it again as a member of the public and not as a cynical critic.

G. MARKS

Harrogate, UK

SIR—In your editorial, "That was the weak force, that was" (3 February 1977), you make a strong criticism of Nigel Calder's two-hour BBC2 brainteaser, "The Key to the Universe." You ask, in effect, not only whether it was worth the resources devoted to it, but also whether it might not contribute to the further alienation of many people from science.

These are important questions to which we have no answer. In this regard the BBC fails us entirely. We are not told how much in financial terms such a two-hour programme costs; we do not know whether the key criterion of 'worth' may not be measured solely by audience ratings; and we are completely in the dark about the effect of such programmes on the general viewing audience. That Calder's book of the same title is high in the *Sunday Times* best-seller list tells us nothing about the TV programme except that it surely helps to sell the book.

The non-scientists with whom I watched the programme confessed that apart from the presentation of the 'black holes,' they were baffled. I found the jargon familiar, but I disagreed with some of Calder's emphases. I think he made too much of the tentative, of the as-yet unaccepted, as in Salam's exposition for example. In that sense, it was difficult to distinguish the 'real' from the 'unreal.'

I have said before that the populariser of science, as he functions today, cannot disseminate the subtle ideas of science; that these really cannot be understood without hard, disciplined effort. We need a research programme on this thesis. The BBC (and ITV) might consider funding that part of it concerned with the presentation of science on TV and radio. Such a proposal concerns the key question of what the presenters of science are really providing, not only in terms of good, entertaining viewing, but also in their contribution to general understanding *en route* to involving thep ublic in decision-making about the use and impact of science in everyday life.

MAURICE GOLDSMITH

Savile Club, London

Tidal energy

SIR—Your leading article (10 February 1977) refers to the 'severe defect' of the periodicity of the tides being not always in phase with the daily load cycle, but nearly 40% of our electrical energy is used for space and water heating where the 'defect' is not at all severe. The use of blocks of tidal energy produced in regular and predictable amounts, both in time and quantity, is now perfectly feasible in the British system, and as installed capacity increases (if indeed it does increase), will become easier rather than harder to absorb. This is without the addition of any further pumped-storage capacity.

The reasons why the French have not proceeded with the Iles de Chausey scheme have nothing to do with the foregoing point, since La Rance incorporates a pumped-storage capability to provide power capacity in phase with peak-demand—if that is what the operators require. (Incidentally La Rance is presently using peaking system energy to pump on those occasions when so doing produces more energy overall.) The reasons are both economic and oceanographic, since the inclusion of sophisticated pumped-storage facilites made La Rance so expensive as to discourage further investment in tidal energy, coupled with fears that a much larger scheme might have reduced the tidal range.

The defects of pumped-storage in estuarial schemes are primarily cost, since such facilities can be provided more cheaply on land (as at Ffestiniog or Dinorwic) or underground, and secondly because the important function of system spinning reserve is unavailable from a tidal pumped-storage plant without loss of available energy. The arguments are all developed in the technical literature and it behoves your leader writer to give it more than a cursory examination before committing himself to a particular solution.

The proposed Severn scheme your article describes is one of at least six proposals made in the last few years, all of which deserve examination in the light of system requirements. It will do the cause of tidal energy no good if a scientific journal of your reputation appears to be prejudging the solution to what is a most complex problem.

E. M. WILSON

Department of Civil Engineering
University of Salford, UK

Geothermal electricity

SIR—Recent studies of potential UK geothermal heat sources (for example, Oxburgh *Nature* **262**, 526, 1976) have been virtually unanimous in stating that temperatures greater than 200 °C would be required from sources intended for electricity generation. A report produced by Patscentre International has shown that, contrary to this general view, it may well be possible to accept a much lower temperature limit. If it can be combined with a fossil-fuel source in an existing or purpose-built power station, geothermal heat in the temperature range of 100–200 °C could be economically used for electricity generation.

In modern steam plants most of the feedwater heat is supplied by low-

Correction

A bad telephone line helped to produce three errors in the article 'Give us a call, says NASA' (10 March, page 112). The Viking chief project scientist is Dr Gerald A. Soffen, not Dr Soffes; the Kioto meeting will be in the first week of April, not in June, and will be organised by the International Society for the Study of the Origin of Life; and the Tel Aviv meeting in June is the COSPAR and not the pulsar meeting. Sorry.

temperature steam from the turbines. However, this steam, which in a conventional station has been heated by a high-grade fossil fuel, is diverted from work-producing duties in the turbine. If low-grade geothermal heat is used for feedwater heating, the steam could be retained in the turbine, thus increasing both the work output and the cycle efficiency relative to the fossil fuel. The resulting capitalised fuel saving (for the normal rated output) can then be used to pay for the necessary geothermal plant. (I have discussed a similar use for low-grade waste heat in MHD power generation in *Energy Conversion* **8**, 177; 1968.) The amount of geothermal heat that would be usable in this system, which could be applied to either an existing or a purpose-built station, is no more than about 15% of the total heat input; that is, about 200 MW(t) could be accepted by a 500 MW(e) steam plant using geothermal heat at 125 °C. This would give a fossil-fuel saving of 5%.

An alternative purpose-built combined plant, in which the geothermal heat predominates, would use fossil fuel for superheating only. In this case, efficiencies in the region of 100% (relative to the fossil fuel) become possible. For example, 100 MW(e) output could be obtained from 100 MW(t) fossil fuel combined with 360 MW of geothermal heat at 185 °C. This method also has the advantage of low steam wetness, thus appreciably reducing turbine blade erosion, which could occur in a plant that is predominantly geothermal.

The cost benefit analysis proceeds as follows. The present saving on fossil fuel is determined from an annuity-type calculation, taking into account, among other factors, the current fuel cost, inflation of real cost of fuel, interest rate, plant life-time, and increased load factor allowed by increased efficiency. Thus a capital allowance (per kilowatt of geothermal power) is obtained which can be compared with estimated specific costs of geothermal plant for various conditions.

Results for a purpose-built plant based on a modern conventional power station illustrate the operation of the first system. With the plant situated on hot dry rock supplying feedwater heat at 125 °C, a fossil fuel cost of 9p a therm, and a load factor of 80%, the capitalised fuel saving over 20 years, with a basic interest rate of 10% a year, is £62 per kilowatt of geothermal power. This equals the (1974) cost of the associated geothermal plant (well-head equipment and so on) calculated from fairly conservative data supplied by the Energy Technology Support Unit (Harwell) for a site having a temperature gradient of 45 °C per km. For a similar station situated on an aquifer, the available cost of £62 per kW(gt) would pay for geothermal plant in the upper half of the range of calculated aquifer costs. Of course, the capitalised fuel saving increases with the fossil-fuel cost, the fuel inflation rate (in real terms), and the load factor. Figures for the second type of plant, using 185 °C geothermal heat, show that its unit electricity costs are comparable with those of conventional power stations.

The Patscentre study has shown that geothermal sites that coincide with, or are accessible to, fossil-fuel sources could be of special interest for electricity generation. For instance, the nearness of the Durham hot rocks (granite) to the Durham coal fields should be noted, and coastal sites on the Hampshire sedimentary basin could also be of interest.

R. V. Harrowell

Patscentre International, UK

Management courses

Sir,—I have long felt it appropriate that the likeness that accompanies Kenneth Mellanby's articles should be as black and white as the views he expresses! However, the truth about management courses for scientists may not be quite as black as his article (3 March, page 11) might suggest. Five years ago A. J. Lockwood and I started a course in research management (*R&D Management* **5**, 235–238; 1975) for heads of research sections at the Building Research Establishment. It has been held annually since then and now draws participants from a wide range of government research laboratories (both civil and defence). Those who attend appear to find it helpful.

The course was started because many section heads felt the need for some help with the management aspects of their work, a need that was understandably not met by the more general management courses that were then available—and it may be that Professor Mellanby's strictures were directed at such courses. Our courses are deliberately designed for those leading research teams Perhaps the secret of whatever success the courses have achieved lies in the very fact that we deliberately refrain from any attempt at 'training'. Rather, we try to make available to participants a wide range of conceptual material and practical experience on which they can draw for use in their own circumstances. More important still, we devote about half the course time to fostering, within a carefully thought out series of syndicate exercises, the exchange of the management experience that participants will have accumulated during their working lives. Just as scientists profit considerably from an exchange of their scientific experience, we have found that they can and do receive considerable benefit from an exchange of their (often unrecognised) management experience.

K. Alsop

Building Research Establishment,
Garston, Watford, UK

Competition 12.
A chair in bio-aeronautics has recently been established in the UK. Its occupant is an expert in crop spraying. £10 for the best real or fictitious professorial title. Entries by 20 April to Competition 12, *Nature.*

Competition 11. Readers' contributions offered humorous associations with the work of scientists. Thus: 'Faraday was incapacitated', and so on through a deluge of awful puns. Jim Watson, according to most competitors, had a guess-what in his neck. Two winners, one brief, one less so.

Brahe thought the paper by Copernicus revolutionary, but pointed out that his argument was essentially circular (M. C. Thorne, Harwell, UK).

On the first day Rutherford reported an immediate disintegration of the Symposium catalysed by Brønsted's acidic comments on Madame Curie's radiant attire. Drs Barnard and DeBakey were further disheartened when Van de Graaff generated a separate reaction from Schiff by referring to her attire as basically colourless. Bragg viewed things from another angle as did Roentgen who saw through it all Compton recoiled at the prospect of making any statement while Planck and deBroglie also went to some lengths not to make waves—an attitude that Moseley called uncharacteristic. Wegener asserted that if the rifts continued to grow things would drift further apart. As usual, Heisenberg was uncertain but Coulomb and Nobel were positive that such a charged situation was potentially explosive. Kelvin and Boyle advised that a cooling-off period might reduce the pressures that were building up Bell thus called on Hubble and Doppler to focus their attention on shifting the emphasis to a series of higher-level discussions as earlier suggested by Balmer and Lyman. Newton seconded the motion and, being short of time, Einstein tried to apply a constant and massive attempt to bring the entire matter to a close. The following day Watson and Crick happily reported that things were taking a turn for the better. (K. M. Towe, Smithsonian Institution, Washington, USA).

news and views

Molecular charmonium?

from Frank Close

A YEAR ago most high energy physicists were agreed that charmed particles must exist, the only problem was that nobody could find any. Then in a study of the debris produced in annihilation of electrons and positrons, charmed mesons called D (1.86 GeV in mass) and D^* (2 GeV) were at last found in the spring of 1976 (see *Nature* 262, 537). Once it was known what had to be looked for and where it could be found old data were studied again and new experiments performed. We now have the surprising result that sometimes too much charm is being found, specifically that when electrons and positrons annihilate at just above 4 GeV an anomalously large production of pairs of charmed D^* mesons is seen. What makes this so remarkable is that the energy of the colliding beams is only just sufficient to produce a pair of 2 GeV mass particles. It is as if the moment that the energy was sufficient to produce them, they just poured out. Why should this be so?

A group of workers at Harvard (de Rujula *et al. Phys. Rev. Lett.* 37, 398; 1976) had earlier suggested on statistical grounds that the relative production rates of $D^*\bar{D}^*$:DD^*:$D\bar{D}$ should be 7:4:1 (there are more degrees of freedom for high spin particles and so the spin 1 D^* is more readily produced than the spinless D). The observed rate of $D^*\bar{D}^*$ production is far in excess of this and so it seems that there is something in the underlying dynamics of the production process that is enhancing the D^* pairs.

When this discovery was reported at a seminar at Harvard, two groups of theorists suggested that charmed molecules might exist (de Rujula *et al. Phys Rev. Lett.* 38, 317; 1976; P. Cox *et al* Harvard preprint, 1976) Their idea was that in the electron–positron annihilation, in addition to resonances of charmed quarks and charmed antiquarks (like the ψ meson at 3.1 GeV mass) there might be found resonances of charmed mesons and their antiparticles. If such a $D^*\bar{D}^*$ resonance occurs at 4.03 GeV, then this could explain the copious production of D^* and $\bar{D}^*$ at this energy.

But critics say that this is tautology. To say that there is a $D^*\bar{D}^*$ resonance is simply equivalent to saying that the $D^*\bar{D}^*$ are produced more often than expected. Since no completely satisfactory mechanism has yet been given for producing such a resonance the copious production of D^* pairs has still not been explained. Supporters argue, however, that the idea can be tested and so has some merit. If energies away from 4.03 GeV are looked at the D^* should be less copiously produced.

Whether or not the idea is right it has suddenly gained wide attention in the high energy community although it is by no means new. Several papers have appeared during the past two years and been largely ignored. They have implied or explicitly suggested the existence of charmed molecules as the consequence of investigating the broader issue of the binding of quark states, and it is in connection with this broader issue that the above discoveries might turn out to be significant.

Multiquark hadrons

Mesons are formed when a quark (q) and an antiquark ($\bar{q}$) bind together with orbital angular momentum (L). The mass of the resulting meson can be estimated from the rough rule that each quark or antiquark contributes around 300 MeV and each unit of L-excitation gives around 500 MeV. The quark has spin 1/2 and so the quark and antiquark can spin parallel (spin 1) or antiparallel (spin 0). Then, just as in atomic physics, the spectrum has the form $^1S_0$, $^3S_1$; $^1P_1$, $^3P_{0,1,2}$ and so on, and a hyperfine splitting causes the singlet states to be lighter than the triplets (for example, the pion $^1S_0$ is lighter than the ρ $^3S_1$)

These rules mean that the lightest vector particles will be around 700 MeV since they are the $^3S_1q\bar{q}$ states. Of particular interest are the scalar states $^3P_0$. From our rule these states will have a mass around 900 MeV since the hyperfine splitting has pulled them down relative to the average mass of about 1,100 MeV for the P-wave $q\bar{q}$ system. Hence roughly half of the mass has arisen from the two quarks intrinsically and half is from the P-wave excitation.

One can also form scalar states (0^+) from $qq\overline{pp}$ where now all of the quarks are in S waves. The extra mass of 600 MeV associated with the two additional quarks is compensated for by the absence of the P-wave excitation and so the mass of this four quark scalar meson can be comparable to the two quark state. In fact the hyperfine splitting in the four quark state tends to be larger than in the two quark and an explicit calculation by R. Jaffe of MIT (*Multiquark hadrons*, SLAC Publications, 1772, 1773) found that the $qq\overline{qq}$ 0^+ is lower in mass than the $q\bar{q}$. Astonishingly he presented a good case for believing that the familiar 0^+ mesons are really four quark states and not $q\bar{q}$! The implication is that four quark states have to be taken seriously.

For states containing a charmed quark (c) the same rough and ready rules seem to apply except that one has to allow 1.5 GeV for each charmed quark instead of 300 MeV. The lightest mesons $c\bar{q}$ will be expected around 1.8 GeV and the lightest charmonium states $c\bar{c}$ around 3 GeV. Both of these are realised in the data. Furthermore the P-wave $c\bar{c}$ states $^3P_{0,1,2}$ do seem to spread around 3.5 GeV in line with the rules.

Now replay Jaffe's game but with charmonium. The 0^+ ($c\bar{c}q\bar{q}$) state could be as low as 3.5 GeV (comparable to 0^+ ($c\bar{c}$) at 3.4 GeV). It is possible that a mysterious state at 3.46 GeV could be such a beast. The states that will be produced in electron–positron annihilation are vectors (spin 1, negative parity). If made from four quarks then a P-wave excitation is required and hence the mass rule implies another 500 MeV compared with the S-wave $c\bar{c}q\bar{q}$ (3.46 GeV?) or 800 MeV above the S-wave vector $c\bar{c}$ at 3.1 GeV. Either of these estimates suggests that the 4 GeV region is the place to look.

Very soon after the ψ was discovered Iwasaki in Japan suggested that $c\bar{c}q\bar{q}$ states might exist and even predicted a $c\bar{c}c\bar{c}$ at 6 GeV. The possible existence of such a state is controversial. When the plethora of dips and bumps were discovered in the 4 GeV region of the e^+e^- annihilation cross section, Rosenzweig (*Phys. Rev. Lett.* 36, 697; 1976) and also

Bander *et al.* (*Phys. Rev. Lett.* **36**, 695; 1976) independently suggested that four quark states $c\bar{c}q\bar{q}$ had been manifested. At the time of these works the charmed states $c\bar{q}$ had not been isolated!

After the D and D* charmed particles had been found with masses around 2 GeV, Nussinov and Sidhu at the Fermi National Accelerator Laboratory near Chicago explicitly investigated the possibility that charmed mesons might bind together in a manner analogous to the way that nucleons bind to form deuterium. Their explicit calculations (Fermilab preprint, August 1976) indeed showed that a loosely bound $D\bar{D}$ state could exist near to 4 GeV and they speculated that a similar occurrence might also happen for DD* and $D^*\bar{D}^*$. In the light of the recent developments a report on their calculations of the $D^*\bar{D}^*$ possibility is eagerly awaited.

If $c\bar{c}q\bar{q}$ states are indeed around the 4 GeV mass region then several questions come to mind. Once formed how do they collapse? Is the decay to $c\bar{q}$ and $q\bar{c}$ preferred to $c\bar{c}$ and $q\bar{q}$? The latter decay pattern has been called "charm burning" and has been investigated in particular by Okun and Voloshin in the Soviet Union. There is no obvious presence of $\psi(c\bar{c})$ in the debris produced at 4 GeV so this latter mechanism may be unimportant. Is this a problem? In the absence of dynamical information on the binding of the four quark states this is perhaps an open question. Are the four quark states (cc)$(q\bar{q})$ necessarily the same as $(c\bar{q})(q\bar{c})$?

If the quark model is relevant to nature then these four quark states seem to be unavoidable. With all the current interest in the 4 GeV region of energy in electron–positron annihilation one might hope that the first evidence for them may show up here. If the ideas of Iwasaki, Rosenzweig, Bander and many others are right, then it is possible that such a state has already been seen, in which case a whole new spectroscopy of molecular charmonium awaits discovery in addition to the "traditional"(!) charmonium now being unravelled. □

Versatile polysaccharides

from R. D. Preston

THE recent explosion of information on the detailed structure of a variety of polysaccharides, largely at the hands of four groups of workers, provides a salutary reminder, for those who need it, that these substances are to be reckoned with as well as proteins and nucleic acids. Broadly speaking, polysaccharides serve two main functions in living organisms. They largely determine the mechanical properties of supporting tissues, such as cell walls in plants and connective tissue in animals, and on the other hand, serve as recognition and receptor sites, a function particularly well-studied in bacteria, in which polysaccharides may be involved in recognition and adsorption of bacterium and phage, and of bacterium and host.

It is this function of polysaccharide that has attracted the attention of two groups of workers, D. A. Rees and colleagues in the UK and S. Arnott and coworkers in the USA. Two recent publications present the first detailed conformation of branched polysaccharides and give structural information relevant to their function as agents of recognition (Moorhouse *et al. J. molec. Biol.* **109**, 373; 1977; Morris *et al. J. molec. Biol.* **110**, 1; 1977).

Structural information on polysaccharides has been scanty until comparatively recently because no polysaccharide gives enough X-ray evidence to allow a complete determination of structure. The recent spectacular advances stem, not from advances in the technique of X-ray analysis itself, but from a combination of X-ray data with conformation theory as developed by Ramachandran and his colleagues and modified particularly by Arnott and his group (Ramachandran *et al. J. molec. Biol.* **7**, 95, 1963; Arnott & Wonacott *J. molec. Biol.* **21**, 371; 1966; Arnott & Scott *J. Chem Soc. Perkin* 324; 1972; Arnott *et al. J. molec. Biol.* **90**, 253; 1974). Both Moorhouse *et al.* and Morris *et al.* use this approach in defining the structure of their polysaccharides and, with the former, in presenting computer-drawn models complete with the necessary hydrogen bonding within and between the molecular chains and the positions of included water and anions.

Moorhouse *et al.* deal with a capsular polysaccharide from *Escherichia coli* strain Bi161/42(09:K29(A):H) in the form of chains of hexasaccharide repeating units [-2-α-mannopyranose-(1 → 3)-β-D-glucopyranose-(1 → 3)-β-D-glucuronic acid-(1 → 3)-α-D-galactopyranose-(1 →] with a disaccharide [β-D-glucopyranose-(1 → 2)-α-D-mannopyranose] attached by a 1 → 4 link from the mannopyranose residue to the glucuronic acid. Pyruvate is attached to every terminal D-glucose residue. This polysaccharide has been isolated from the serotype K29 and from two mutants, M13 and M41. The structure turns out to be the same for the mutants as for the wild type. The unit cell of fibres maintained at 92% R.H. is orthorhombic with dimensions a = 2.030 nm, b = 1.178 nm, c (fibre axis) = 3.044 nm. This contains four hexasaccharide units, one along each c edge and one central, and the space group $P2_12_12_1$ dictates that the central chain lies antiparallel to the side chains. The conformation of the chain is presented in Fig. 1. The main chain lies in a two-fold helix and the side chains are folded back to lie parallel to the main chain. Of the 15 potential hydrogen donors in each asymmetric unit, only five have been explicitly identified as participating in hydrogen bonds within and between chains. Presumably the other 10 may represent hydrogen bond bridges utilising a single water molecule between the chains. When the fibres are dried over silica gel, the unit cell is reduced in volume by contraction in a (to 1.73 nm) and b (to 1.02 nm) and then several of these bridges appear to be converted into direct intermolecular hydrogen bonds. *In vivo*, this highly hydrated polysaccharide is attached to the exterior of the cell in all three forms either as a capsule or contributing to long loose strands which project outwards.

Its functions seem to be many. *E. coli* phage 29 is highly specific for this capsule, cleaving β-D-glucosido-(1 →3)-D-glucuronic acid bonds exclusively and is dependent on the presence of the pyruvate ketal. The authors consider that the viral glucanase may move along the polymer strands, cleaving the glycosidic bonds and thereby opening a path for the phage head. Some particular conformational aspect of the polysaccharide, still to be determined, may then direct the phage to a particular site. Otherwise, the capsular polysaccharide prevents bacteriophages specific for cell wall receptors from infecting the cell and protects the bacteria from phagocytosis by macrophages; the structure so far elucidated gives no clue to the molecular mechanisms involved.

Although Morris *et al.* (*op. cit.*) refer to the structure determination of the polysaccharide they discuss, the details have not yet been reported and their paper deals exclusively with the behaviour of the chain molecules in solution, as judged by temperature effects on optical rotation, circular dichroism, proton magnetic resonance and viscosity; to this extent it complements the findings of Moorhouse *et al.* They deal, however, with different polysaccharides, the extracellular polysaccharides of *Xanthomonas* spp., pathogens responsible for blight disease of a number of important crop plants. These have a range of distinctive rheological properties which give them a variety of industrial uses. The primary structure consists of a backbone of repeating [→4)-β-D-glucopyranose-(1 →4)-β-D-glucopyranose(1 →] as in cellulose, with a substituent [3 →1)-α-D-mannopyranose-6-O-acetate-(2→1)-β-D-glucuronic acid-(4→1)-β-D-mannopyranose] on every other glucose residue. Each side chain appears to carry O-acetate, but in addition pyruvate is found attached to mannose residues though only to about one third of this extent.

The crystallographic evidence (not yet reported) is said to imply that the side chains, like those of the *E. coli* polysaccharide, fold down to form favourable noncovalent interactions with the main chain, and the molecule as a whole constitutes a five-fold helix in the form of a rigid rod. All the spectroscopic evidence with solutions points to a change from an ordered form to a disordered form as the temperature is raised. The transition temperature is independent of concentration and from this it is argued that the ordered form is a single-chain species (though it is agreed that this is not yet completely certain) and that this ordered form could have the same conformation as in the fibre This last consideration is one that vexes all attempts to understand solution properties, and properties *in vivo*, in terms of the conformation of the solute molecule when in a crystallite, and it cannot be said that the authors give direct proof of this correspondence. If the correspondence is acceptable, then the rheological behaviour can be understood in terms of weak interactions between rigid rods forming a tenuous three-dimensional network which is dispersed as the temperature rises.

A clue to the function of the polysaccharide of this pathogen is given by the observation that when mixed with even low concentrations of galactomannan the mixture may gel even though neither polysaccharide alone does so. The interaction is thermally reversible, and the gel is stronger when the ratio of mannose to galactose is 4:1 than it is at a ratio of 3:1, and when the ratio is 2:1 the mixture does not gel at any temperature. From this it is deduced that noncovalent binding occurs between the unlike chains of 'cellulose' and mannan. This could be regarded as 'recognition' of one polysaccharide by another. The interaction is not confined to galactomannans, however, but occurs with other polysaccharides with the β-1, 4-linked backbone including cellulose derivatives (Dea, Morris & Rees, in the press). There are therefore in the walls of the host plant a number of polysaccharides to which the bacterial polysaccharide might become attached and the suggestion made by the authors that this might constitute a molecular 'holdfast' securing the pathogen to the cell surface is not without its attraction. In going further by suggesting that this mechanism might ensure recognition of particular sites on particular plants the authors are, however, clearly going beyond the evidence. There seems no reason at the moment to believe that this association between polysaccharides would confer sufficient specificity and further evidence is needed on the polysaccharide composition of cell wall surfaces in host and non-host plants before accepting this as 'recognition'.

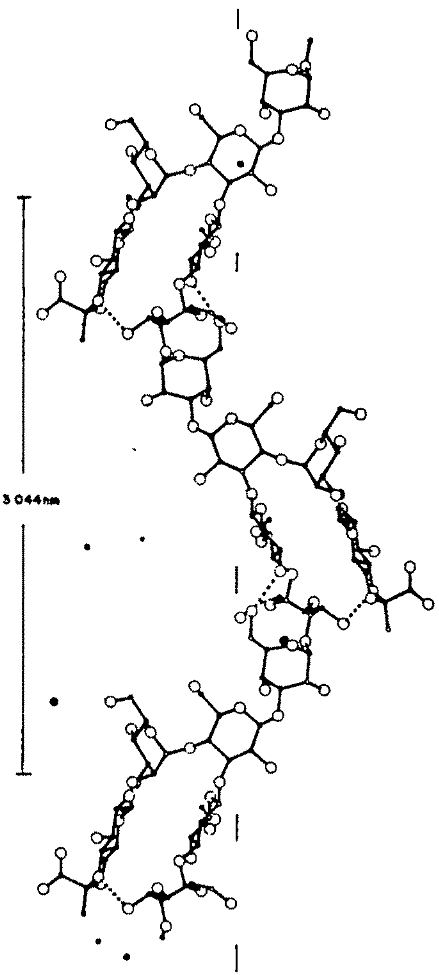


Fig. 1 Molecular conformation of the M41 polysaccharide. Open circles, oxygen; hydrogen omitted. Broken lines denote hydrogen bonds.

It is welcome that two groups of workers have now delineated the conformations of two branched polysaccharides and that, in spite of the different chemistry, there is an overall similarity between them. This will one hopes stimulate closer attention to the other partner in the proposed recognition mechanisms, namely to the possibility of variations in the matrix substances of the walls of host and non-host cells, and, for *E. coli*, to the structure of the viral glucanase of phage 29 as a check on its compatibility with the function claimed. More remotely from the field of the present two papers, but of equal urgency, success with these two polysaccharides should stimulate attempts at the conformation of branched polysaccharides in higher plant cell walls, to the benefit of cell wall mechanics both in cell growth considerations and in, for example, timber technology. The importance of these papers ranges well beyond the confines of bacterium/host recognition.

Laser dissociation

from Graham Richards

LASERS offer to chemists the possibility of controlling the specificity of chemical reactions by choosing the energy of the laser beams so as to cause the rupture of particular chemical bonds. Most attractive from this point of view is the prospect of isotopic separation by laser dissociation of polyatomic molecules. Powerful CO_2 infrared lasers have been used successfully to produce enrichment of boron isotopes from BCl_3, sulphur from SF_6 and more recently amongst heavier nuclei, osmium from OsO_4 (Ambartzumian *et al.*, *JETP Lett.* **22**, 43; 1975) and molybdenum from MoF_6 (Hudson *et al.* *Electro-Optical Systems Design Conference*, 1976). The hope is that the technique may lead to a method of separation of uranium isotopes by laser action on UF_6.

The dissociation of a molecule by infrared radiation, whose photons have enough energy to increase vibration but far too little to break a bond, can only succeed if many photons are absorbed so rapidly that energy is not lost on collision between molecules. The high power density of the CO_2 laser enables these conditions to be met. The sharp frequency of the laser may fit with a vibrational frequency of one isotopic species, but not with another since in molecules, as in all vibrating systems, the vibration frequency decreases with increasing mass of the oscillator. Once started up the vibrational ladder by absorbing several photons, one isotopic molecular species may be decomposed leaving an isotopically enriched mixture.

Despite a great deal of work, many questions about the mechanism of this potentially valuable technique remain. How can a molecule absorb a number of quanta of the same energy even though the vibrational energy levels converge as dissociation approaches? It could be due either to multiphoton absorption (several photons absorbed at the same instant) or to the use of rotational energy levels which give fine structure to the vibrational energy ladder. Theoretically the former hypothesis seems more likely (Bloembergen *Opt Commun.* **15**, 416; 1975), but even in the case most studied, SF_6, the mechanism of multiphoton absorption is far from completely understood. What are the dissociation products; how many photons can a single molecule absorb beyond the dissociation limit, and how is the excess energy distributed among the fragments? What is the effect of molecular collisions and is there an activation energy barrier beyond dissociation? Many of the unanswered questions are clarified by the elegant experiment of Coggiola *et al.* (*Phys. Rev. Lett.* **38,** 17; 1977) on the multiphoton dissociation of SF_6. They establish that the molecule does accumulate energy by sequential absorption of photons and provide many details of the mechanism.

Their experiment involved crossed molecular and laser beams; an SF_6 beam formed by expansion was crossed with a pulsed CO_2 laser beam (1 J per pulse). The fragments produced by multiphoton dissociation of the SF_6 were analysed with a quadrupole mass spectrometer whose position could be varied to measure the angular distribution of fragments.

The observation of dissociation products from the beam interaction region confirms that the multiphoton molecular dissociation is indeed a unimolecular process since there is no possibility of collision.

The SF_5^+ ion signal is always the most intense and no F_2^+ was detectable. Ions with one, two or three fluorines removed and F^+ all have the same parent molecule, SF_5. The major products are thus SF_5+F, rather than SF_4 and F_2. The possibility that clusters or van der Waals dimers of SF_6 are the origin of the fragment signals was ruled out by studying the dependence of the SF_5 signal as a function of SF_6 beam saturation pressure when no increase in intensity beyond that due to increased beam flux was seen.

The translational energy released in the dissociation was found from the angular distribution of products using conservation of energy and linear momentum. It proves to be very small (< 6.3 kJ mol^{-1}), implying that there is no substantial barrier beyond the dissociation limit. The activation energy required beyond dissociation is less than 70 cm^{-1}.

The laser affords the possibility of varying intensity, power, polarisation and wavelength, and the SF_6 beam may be excited both vibrationally and rotationally. Further experiments should thus yield detailed information about dissociation dynamics and internal energy distribution and energy transfer of an excited molecule before dissociation. This should in future give a much better understanding of the mechanism of multiphoton dissociation. □

Specificity of chromatin transcription

from Stewart Gilmour

MUCH of the information about gene regulation in animals has come from experiments in which chromatin is transcribed *in vitro* with exogenous RNA polymerase. In many cases *Escherichia coli* RNA polymerase is used and it is pertinent to ask whether the transcriptional patterns reflect the regulatory features of chromatin or an incompatible marriage of bacterial polymerase and animal chromatin. Could it be that a bacterial enzyme may not recognise some active sequences in chromatin or perhaps transcribe other sequences which are normally inactive *in vivo*? An insight into this question has been provided recently by Parker and Roeder (*Proc. natn. Acad. Sci. U.S.A.* **74,** 44; 1977) for the particular case of 5S RNA gene transcription in isolated *Xenopus* oocyte chromatin. Normally *in vivo* these reiterated genes are transcribed by RNA polymerase III and in immature oocytes this accounts for a substantial proportion of the total RNA synthesis. The spacer region between the gene copies is apparently not transcribed.

In these experiments chromatin isolated from immature *X. laevis* oocytes was transcribed *in vitro* with exogenous RNA polymerase I and III from *X. laevis* ovaries, polymerase II from mouse plasmacytoma cells and *E. coli* RNA polymerase. The authors devised a hybridisation assay which analysed the fidelity of the chromatin transcripts with respect to sense strand, anti-sense strand and spacer region copying of the 5S gene. This was achieved by two separate hybridisations. First, transcripts labelled with ^{3}H–UTP were hybridised to plasmid DNA containing homologous *X laevis* 5S DNA in the presence and absence of saturating amounts of unlabelled 5S RNA. The levels of hybridisation in the two cases indicated the degree of sense and antisense strand copying and by simultaneous inclusion of standard ^{32}P–labelled 5S RNA in the hybridisation reactions an accurate estimate could be made of the total 5S RNA transcript synthesised. In the second analysis transcripts were hybridised to plasmid DNA containing 5S DNA from *X. mulleri.* Since only the 5S gene regions and not the spacer regions are homologous between *laevis* and *mulleri* any aberrant transcription of spacer will not score as hybrid in this case.

Incubation of *X. laevis* chromatin without additional polymerase reveals endogenous polymerase III activity. About 40% of the transcript is 5S RNA transcribed from the sense strand. In control experiments where only ^{3}H–UTP is present some terminal addition to 5S RNA occurs and an appropriate correction has to be made. The addition of exogenous polymerase III results in a <50-fold increase in 5S RNA synthesis; the percentage of 5S RNA in the transcript is about the same as for the endogenous activity and is asymmetric. In contrast exogenous polymerase I did not stimulate 5S synthesis even though there was a net stimulation of total RNA synthesis above endogenous levels. In all cases a small but indeterminate amount of spacer transcription is seen. It is clear that the transcription of 5S RNA from *Xenopus* chromatin requires the mediation of a specific polymerase. This conclusion is reinforced when chromatin is transcribed with mouse plasmacytoma polymerase II and *E. coli* RNA polymerase. In neither case is there a specific stimulation of 5S RNA synthesis.

In another series of experiments purified *X. laevis* DNA was transcribed in identical conditions with *Xenopus* I, III and *E. coli* polymerase. In each case only about 1% of the transcript was 5S RNA and copying was symmetrical. Taken together these findings suggest that chromatin-associated proteins are required for the selective and asymmetrical transcription of 5S RNA genes in amphibian oocytes and that a specific polymerase is required to effect this. It is clear from the data that a high degree of proper initiation and termination occurs. The situation with other cell-free systems is unfortunately not so precise. A number of laboratories are currently investigating the control of specific genes in a variety of animal systems (such as globin, ovalbumin and histone genes) by analysing the *in vitro* transcripts from isolated chromatins.

In nearly all cases *E. coli* RNA polymerase rather than the homologous polymerase II is used because of the relative inactivity of the latter when added exogenously. The transcripts are usually analysed by hybridisation to complementary DNA made from the appropriate mRNA. It could be argued that the analysis is geared to the biological expectation of the template rather than to the fidelity of the polymerase, however it is clear from these experiments that bacterial polymerases can transcribe chromatin in a tissue-specific fashion. The question of how specific the overall transcription process is in these systems has been studied recently by Biesmann *et al.* (*Biochemistry* **15,** 4356; 1976) by transcribing *Drosophila* chromatin with *E. coli* polymerase and hybridising the RNA to cDNA made to the polyadenylated nuclear RNA of the tissue. In summary these results showed that while most of the sequences represented in the cDNA probe were also found in the transcript some aberrant transcription also occurred. While it is possible to demonstrate a fair degree of the *in vivo* transcription pattern *in vitro* by this approach it has to be conceded that some genes, like the 5S gene, may be subject to finer levels of control which may not be recognised by heterologous polymerases. Gross controls such as heterochromatisation, acting at the structural level, might determine which tissue-specific genes are transcribed from chromatin *in vitro* and may require little discrimination on the part of the polymerase for the selective transcription of these genes.

Triassic extinction or Jurassic vacuum?

In his article "Triassic extinction or Jurassic vacuum?" (*News and Views* **265**, 402; 1977), Milner refers briefly to my work on a Middle Jurassic microvertebrate locality in Oxfordshire. (*Science* **194**, 1053; 1977). He expresses hope that it and other new localities in Queensland, India and the Isle of Skye may provide data on the times of extinction of the procolophonids, rhynchosaurs, thecodonts, dicynodonts and temnospondyl labyrinthodonts, all of which are conventionally supposed to have died out at the end of the Triassic.

The Oxfordshire site has yielded isolated bones and teeth of mammals, therapsids, theropods, ornithischians, ?sauropods, pterosaurs, crocodilians, lepidosaurs, chelonians, anurans and actinopterygians, but in spite of a deliberate search, no teeth of labyrinthodonts have yet been found. The fragmentary condition of the material would not permit ready identification of the other Late Triassic groups to which Milner refers even if these were present. The diversity of the vertebrate fossils from Oxfordshire indicates that a wide range of vertebrate palaeoenvironments fell within the catchment area of the deposit, and that therefore the absence of the labyrinthodonts in the Late Bathonian is real rather than apparent.

Eric F. Freeman

Difficulties in solving heparin structure

from Edward A. Johnson

Heparin has now been in clinical use for 40 years, but the achievement of anything like a complete understanding of its anticoagulant action still seems a long way off. The difficulties centre on our ignorance of the structure of heparin; or perhaps one should say structures, for it is clear that not only is the most thoroughly purified material a mixture of many compounds, but various components of the mixture exert different effects within the coagulation 'cascade'—that insecure sequence of enzyme reactions which may sometimes begin with the activation of Hageman factor and (with more confidence) may be said to end with the cross-linking of fibrin by activated fibrin stabilising factor to form an insoluble clot.

A matter in dispute since the discovery of heparin is its native form in tissue; more specifically, in the granules of mast cells which are its only certain source, although Jaques and coworkers (*Lancet* **i**, 411; 1977) have recently suggested that it may be present in other kinds of cell. Silbert, Austen and coworkers (*J. biol. Chem.* **252**, 518; 1977) have now consolidated earlier work by Horner (*J. biol. Chem.* **246**, 231; 1971), confirming that, in some tissues at least, native heparin has a high molecular weight of the order of 750,000 to 1,000,000. These large molecules are made up of a number of chains more nearly the size of commercial heparin (5,000–40,000) linked to a common core of uncertain character by polypeptides which, surprisingly, are resistant to all the proteases to which they have so far been exposed. However, treatment with aqueous alkali, a procedure often used in commercial heparin extraction, readily releases the chains from the core to give a product which is presumably the equivalent of the heparin of commerce.

Heparin, as a strongly anionic polysaccharide, exerts its effects by binding specifically to basic regions of certain proteins, in particular to antithrombin III (see Seegers, Rosenberg and other workers in *Adv. exp. Med. Biol.* **52**, 1975), the inhibitory action of which on serine esterases in the coagulation cascade is thereby greatly accelerated. It is reasonable to suppose that binding will depend on the presence of appropriate sequences peculiar to the heparin chains, since related acidic glycosaminoglycans and other polysaccharides, whether artificially sulphated or not, are all far less effective than heparin in the test systems used. The difficulties of identifying these sequences are very great, principally because heparin is always heterogenous, a complete obstacle to the quasi-statistical methods used in sequencing proteins. Also, hydrolytic degradation is invariably accompanied by far more damage to the released fragments than occurs with peptides, and enzymic methods have to be used.

Useful initial fractionation may be carried out by making use of protein–heparin binding, and, by adsorption on matrix-bound antithrombin or by related procedures, several groups of workers (Lam *et al. Biochem. biophys. Res. Commun.* **69**, 570; 1976, Höök *et al. FEBS Lett.* **66**, 90; 1976; Andersson *et al. Thromb. Res.* **9**, 575; 1976) have concentrated heparin fractions with strong antithrombin binding from various commercial preparations. Such high-affinity material has been called, too loosely, 'active' heparin in contrast to the more weakly bound 'inactive' heparin (which seems to be indistinguishable in its general physicochemical properties), but it has yet to be proved that antithrombin-binding capacity *in vitro* is the only significant criterion for anticoagulant activity in heparin. It may be important; but there is certainly evidence that, at least *in vitro*, the antithrombin-binding capacity of certain heparin fractions does not follow their activity in other and presumably unrelated stages in the coagulation cascade (Andersson *et al. Thromb. Res.* **9**, 575; 1976). High-affinity heparin is still very heterogeneous, but Lindahl's group, with Linker (*FEBS Lett.* **69**, 51; 1976), has taken a further step by adsorbing heparin fractions on antithrombin–Sepharose and incubating the complex with heparinase. The segments of the heparin chains bound to the protein were not attacked, but could be displaced in the usual way by increasing the ionic strength. The material isolated in this way had molecular weights of 2,000 to 5,000 (6–16 monosaccharide units), depending on the heparin starting material, but was still heterogeneous.

This kind of approach, ultimately identifying and comparing the heparin segments that are most strongly bound to various proteins involved in coagulation, is difficult and laborious; few of the other relevant proteins are as easy to isolate or as stable as antithrombin III. Nevertheless, it is probably the best hope so far of gaining a proper understanding of heparin anticoagulation. Additional help can come from fluorescence and circular dichroism effects of heparin–protein interactions and also from X-ray diffraction studies on heparin salts—which may give some insight into heparin conformations in solution—but so far as can be seen the obstacle of heparin heterogeneity must in every case limit the information that can be gained. Any useful procedure should include studies of heparins from different tissues and species; lung and mucosal heparins have been known for many years to differ chemically to a small but consistent extent (do the mast cells differ?) and, since this difference is now known to be paralleled by differences in relative activity at different stages in the coagulation cascade, its significance needs to be examined.

One of the driving forces behind this work has been the importance of heparin in clinical practice, including, especially, its use for antithrombotic prophylaxis following major surgery (see *Heparin*: *Chemistry and Clinical Usage* (eds Kakar and Thomas) Academic, London, 1976). It is generally agreed that in this context, where prevention of venous thrombosis must not be accompanied by a tendency to haemorrhage, antithrombin potentiation, as determined by the so-called anti-Xa assay, is as yet the best criterion of efficacy. A recent observation of Thomas and coworkers (*Lancet* **i**, 120; 1977) is therefore disturbing; it demonstrates that a semisynthetic heparin-like compound, which has negligible anti-Xa activity *in vitro*, nevertheless shows excellent antithrombin potentiation in the human subject. In heparin therapy the correlation of assay results *in vitro* with consequences *in vivo* has been taken on trust perhaps more than in other fields, and this will also need reconsideration. □

Probing facilitated transport

from S. J. Smith

INSULIN acts on fat cell plasma membranes to promote glucose uptake and its stimulation of the utilisation of this sugar seems to be amplified by the production of a 'second messenger'. The glucose transport system in adipocytes has been well characterised as a carrier-mediated facilitated diffusion mechanism which shows bidirectionality, stereospecificity and saturation kinetics. However, little is known about the system in structural terms.

Aryl azide derivatives provide a powerful technique—photo-affinity labelling—for identifying sites of biological importance. Irradiation of the probe causes photolysis of the azido moiety producing a highly reactive nitrene, which can react covalently with any groups that lie within reach. In order to provide any interpretable information, the reagent must be shown to bind at the required locus before photolysis. Singer and coworkers have recognised the possibility of 'pseudo-affinity' labelling, which could occur, where the photolytic intermediate is able to bind reversibly to and dissociate from the active site before a covalent bond is formed. In such cases, the process would be analogous to affinity labelling. Attempts have been made to use this technique to label biological receptors in various systems.

Recently, a photoreactive aryl azide derivative of glucose, N-(4-azido-2 nitrophenyl)-2-amino-2-deoxy-D-glucose has been used to label adipocyte membranes (Trosper & Levy *J. biol. Chem.* **252**, 181; 1977). In the absence of light it has been shown to inhibit the uptake of D-glucose and 3-O-methyl glucose competitively, and conversely, the uptake of the probe is decreased in the presence of glucose. Measurement of the initial uptake of the probe gave values for K_t and V_{max} similar to those obtained for glucose in identical conditions, suggesting that both sugars are being transported by the same system. When intact adipocytes were treated with the radiochemically-labelled derivative, on photolysis only, four hot membrane components could be identified in SDS polyacrylamide gels of the purified plasma membrane fraction. When homogenised material was used most of the membrane proteins become labelled. Two of the specifically labelled components were found to be glycoproteins with apparent molecular weights 100,000 and 81,000. In the presence of glucose, labelling of these components was significantly reduced, suggesting their involvement in the glucose transport system. Two lower molecular weight proteins, located on the external surface of the adipocyte membrane were also modified, but substrate protection could not be demonstrated.

The participation of membrane phosphorylation in the regulation of permeability has been implicated in, for example, toad bladder and rat brain synaptic membranes and two reports suggest that this process may be involved in modulating the effect of insulin on glucose uptake. Cuatrecasas (*J. biol. Chem.* **249**, 3170; 1974; **249**, 6854; 1974) reported that low concentrations of exogenous ATP can inhibit insulin-stimulated glucose transport and that this effect is associated with the phosphorylation of two membrane components of molecular weights 22,000 and 16,000 which can be separated from unlabelled components by SDS polyacrylamide gel electrophoresis. Phloretin, a strong competitive inhibitor of glucose transport, can protect cells from the ATP effect with concomitant reduction in the phosphorylation of both these components. In these experiments the binding of ^{125}I-labelled insulin was unaffected, as was the anti-lipolytic effect of the hormone. In further studies, it was found that in the presence of cyclic AMP, but not cyclic GMP, the same membrane proteins were rapidly phosphorylated, and a Ca^{2+}-sensitive cyclic-AMP dependent kinase was isolated from purified adipocyte plasma membranes. This system could not be found in guinea pig adipocytes, where glucose transport is not sensitive to insulin. The physiological importance of these findings is difficult to assess, particularly as no direct effects of insulin or adrenaline could be obtained by pretreatment of the cells with hormone before purification of the membranes.

However, more recently Avruch *et al.* (*J. biol. Chem.* **251**, 1511; 1976) have shown a significant increase in the phosphorylation of a 26,000 molecular weight component of adipocyte plasma membrane, in the presence of adrenaline (even at physiological concentrations) and it seems that this effect is reversed when insulin is also present. The relation, if any, of this protein to that described by Cuatrecasas is obscure. Higher molecular weight membrane components (79,000 and 62,000) could also be phosphorylated, but the extent of this was not hormone sensitive.

The development of the aryl azide derivative of glucose, which seems to be a potential probe for the facilitated glucose transport system provides an obvious strategy for the next step, determination of whether these phosphorylated proteins are involved in the regulation of glucose uptake. □

Plugging the gap in the astronomical spectrum

from a Correspondent

THE last gap in the electromagnetic spectrum available for observation by astronomers seems likely to be plugged in the near future, with the development of instruments purpose built for operating in the 1 to 10 mm band, between infrared and radio astronomy. One such instrument, with a dish 10 m across, has been proposed by observers at CalTech, where a small test dish has already been built. At the meeting of the Royal Astronomical Society on 11 March R. E. Hills, of the Mullard

Radio Astronomy Observatory in Cambridge described plans for a proposed UK instrument, with a dish 15 m across and designed to operate down to 0.8 mm.

This project—at least as a design exercise—got off the ground 18 months ago when the Science Research Council set up a steering committee, chaired by Anthony Hewish and managed by members of the Appleton Laboratory, with Hills as project scientist. The product now available is a fairly advanced design and specification for an instrument intermediate between a conventional optical telescope and a radio dish, requiring its own technology but incorporating ideas from both those areas.

Hills explained that the lack of observations in the millimetre gap has been due to inadequate technology; the photons in this band each carry too little energy to trigger the kind of detector used in, say, X-ray work, but the wavelengths are small enough to make observation by standard radio techniques difficult. With infrared observers pushing one way—stretching their instruments beyond their original design capability—and radio astronomers pushing the other, the gap has shrunk, and now the technology is available for purpose built instruments to complete the job.

Why should astronomers be so keen to plug this gap, when already today they have access to far more information, across a broader band of the spectrum, than ever before? Primarily, the prospects are exciting because this band corresponds to emission from cold material, at about 10 K, and therefore provides a window to view the very substantial fraction of matter in the Universe that is now known to exist in cold clouds. Star formation regions, cool envelopes surrounding old, evolved stars, the distribution of cold matter across individual galaxies, and the differences between galaxies, could be probed with the new telescope and there is also the prospect of penetrating deeper, in observational terms, into the dusty, active regions at the centre of many radio galaxies and similar objects.

How is this to be achieved? The instrument described by Hills, although larger than any existing optical telescope, would still be housed in a dome, but a dome with a rather large aperture in order for the large dish to have an unobstructed view. This would raise problems with wind blowing into the dome, which might be overcome by providing a screen of material, transparent at millimetre wavelengths, across the 'slit'—something suitable might be sailcloth backed with polythene. The instrument itself, the steering committee suggests, should be parabolic, designed on the homology principal which has proved so successful with some very large radio telescopes. This technique allows the instrument to bend under its own weight as it is moved into different orientations, but the design ensures that it always bends into a paraboloid; the effect is to shift the focus of the instrument, but it is much simpler to move the detectors sited at the focus as necessary than to attempt to keep the whole dish rigid.

Such a 15-m instrument should give 13 arc s resolution (better than single radio dishes; worse than optical telescopes) and would need a pointing accuracy of 2 arc s to make best value of the resolving power. The estimated cost of such a facility is £3.5 million, enough to make the SRC blanch at the present time, no doubt, but not so great as to make this an obviously 'pie in the sky' project. The instrument would, of course, have to be built at a high site free from obscuring water vapour—the criteria are essentially the same as those which make Hawaii the site of the UK infrared telescope—and this would certainly mean that it would be run as a national facility for use by many teams, as large optical telescopes are, and would not be the raison d'etre of a single group, as has generally been the case with large radio observational facilities. There is no doubt that the need for the facility exists, since nothing else now being planned will observe below 3 mm, and since most other planned instruments are restricted because they are to be built inside fixed radomes, with no opening slit. But any project requiring millions of pounds of new money today must have only slender prospects of realisation.

Pluto: the little-known planet

from David W. Hughes

PLUTO, the ninth and most distant planet in the Solar System has been and still is an object of considerable mystery and controversy. In 1930, when it was discovered, people were worrying about what to call it, Minerva and Pluto leading the field. Pluto, the brother of Jupiter and Neptune, won. Today, 47 years later, controversy still rages over its most fundamental physical parameters, its diameter, mass, density, surface covering and spin axis direction.

The first measurement of Pluto's diameter was made by Kuiper in 1950. Observing with the Hale Observatories 200-inch telescope at Mount Palomar during a period of excellent seeing, Kuiper compared the planet's image with that produced by a "disk meter", a device which produces an artificial image of adjustable brightness, colour and diameter. He obtained an angular diameter of 0.23 s of arc equivalent to a planetary diameter of 5,900 km. Unfortunately the systematic errors in such measurements of marginally resolved images are rather large. The fact however that Pluto did not occult a specific star in 1965 gave an upper limit to the diameter of 6,800 km.

An indirect approach to the diameter can be made by measuring the brightness of the planet and the albedo (or reflectivity) of its surface. Cruikshank, Pilcher and Morrison of the Institute for Astronomy, University of Hawaii, used the 158-inch Mayall telescope at Kitt Peak National Observatory to measure the reflectance of Pluto in the near infrared (1.2 to 2.2 μm). They used standard JHK filters and two specially made narrow band filters centred at 1.55 and 1.73 μm. Their results are reported in *Science* (**194**, 835; 1976).

The most likely constituents of the surface of the outer planets of the Solar System are the ices or frosts of water, carbon dioxide, ammonia and methane. Water ice has already been found in the rings of Saturn, on four of Saturn's satellites and also on the Jovian satellites Europa and Ganymede. Fortunately reflectance measurements between 1.4 and 1.9 μm can distinguish between CH_4, H_2O and NH_3 and Cruikshank *et al.* find that the ratio between the flux at a wavelength of 1.55 μm and that of 1.73 μm is especially useful diagnostically. For Pluto this ratio is 1.6 ± 0.1 which they interpret as indicating that CH_4 frost is the dominant reflecting material on Pluto's surface. The observations rule out the existence of large quantities of H_2O or NH_3 frost and of CH_4 hydrates. The CH_4 frost is probably mixed in with silicate or carbonaceous material.

There are two possible sources for this solid methane. First the temperature of the pre-planetary nebula could have dropped below 40 K at Pluto's distance from the Sun leading to the direct condensation of solid CH_4. Standard equilibrium condensation models would then indicate that Pluto has a mean density of 1.2 g cm^{-3}. Second, CH_4 8 H_2O could have condensed out at nearer 70 K, internal heating in the planet leading to the outgassing of CH_4 which subsequently froze on reaching the surface.

The brightness of a planet is proportional to d^2a/R^2 where d is the diameter, a the albedo and R the distance between the planet and the observer. Rocky planets and satellites (Mercury, Mars, Moon) have low albedoes (0.056, 0.16, 0.067), frost covered satellites (Europa, Ganymede, Rhea, Dione and Tethys) have albedoes in the range 0.4 to 0.6. The presence of CH_4 frost on Pluto would put its albedo into this range too. Assuming that Pluto has an albedo of 0.4 and a mean opposition magnitude of 14.90, the diameter would be 3,300 km. An albedo of 0.6 implies a diameter of only 2,800 km. Both these values make Pluto smaller than our Moon (diameter 3,476 km).

But Pluto is not completely covered with CH_4 frost. If it were its brightness would be constant. Between 1952 and 1955 the magnitude varied asymmetrically by about 0.11 with a period of 6 d 9 h 17 min, this being the rotation period of the planet. The brightness fluctuations are caused by successive passages through the meridian of regions of differing reflectivity. Andersson and Fix (*Icarus* **20**, 279; 1973) found from observations made between 1971 and 1973, that the amplitude variation is now larger (0.22 mag) and the mean magnitude fainter (15.1). This is thought to be due to a change in the aspect of the planet as it moves round its orbit. The equatorial plane of Pluto is probably inclined at over 50% to orbital plane and the decrease in brightness is thus due to the sub-Earth point on the planet moving towards the equator during the past 40 yr, the polar regions having a higher albedo than the equatorial regions.

The mass of Pluto has been obtained from observations of the perturbations that this mass induces in the orbital motions of Uranus and Neptune. Seidelmann *et al.* (*Astr. J.* **76**, 488; 1971) obtained, for example, a value of 0.11±0.02 Earth masses (6.6×10^{26} g). However Ash, Shapiro and Smith (*Science* **174**, 551; 1971) found that the value obtained for the mass of Pluto is extremely sensitive to the choice of perturbation data and parameter sets and that this mass cannot be determined with any certainty from this source.

If the density and size of the planet are known the mass can be easily estimated. The surface of Pluto inferred from the observations of Cruikshank *et al.* is exactly that to be expected for a large object which condensed at low temperatures in the outer solar nebula. Its composition is probably dominated by frozen volatiles, just like most outer planetary satellites. (Remember there is a distinct probability that Pluto used to be a satellite of Neptune, but escaped during some encounter with Triton in the early times of the Solar System). If this is so its mean density is likely to be between 1 and 2 g cm^{-3}. Combining these with Cruikshank *et al.*'s values for the diameter (3,300 km if $a=0.4$, 2,800 km if $a=0.6$) gives mass values in the range 1.2×10^{24} to 3.8×10^{25} g, about 1/250 the mass of Earth. This mass is much too small to perturb Uranus and Neptune in a measurable fashion, a conclusion which leads to two interesting consequences. First Clyde Tombaugh's discovery of Pluto must have been to a great extent fortuitous, the result of a diligent search and not an accurate, mathematically-derived, prediction as to the planet's position. Second if Pluto is not responsible for the perturbation of the orbits of Uranus and Neptune, something else must be; another planet or the cometary cloud perhaps? ☐

Palynology at Lucknow

from Peter D. Moore

> The 4th International Palynological Conference was held at Lucknow on 29 December 1976–5 January 1977. The Proceedings are to be published.

OF particular interest at the conference were the accounts given of recent vegetation history in the tropics and subtropics, based upon studies of subfossil pollen grains stratified in peats, lake sediment and salt lake deposits. In contrast to the temperate areas of the world, the lower latitudes have been neglected and their recent environmental history is consequently little known. The work of J. R Flenley (University of Hull) in New Guinea and A. P. Kershaw (University of Canberra) in north-eastern Australia has shown that tropical rain forest has not had the long uninterrupted Quaternary history which was once imagined. On the Atherton Tableland of Queensland, Australia, at an altitude of over 700 m, Kershaw has shown that the vegetation changed gradually from a microphyll vine forest to sclerophyllous woodland at about 25,700 b.p , which could have been due to increasing aridity or to the influence of early man, using fire. The development of rain forest at the site did not occur until about 7,000 b.p.

Within India itself, the work of G. Singh (University of Canberra) and others has been of considerable value in elucidating the history of the deserts of western Rajasthan. The study of dry salt lakes in this area has shown that between 10,000 and 4,000 b.p. the sites were occupied by freshwater lakes and the pollen contained in these sediments demonstrates a development through *Artemisia* steppe to savannah vegetation with trees and shrubs. Desert encroachment followed this phase and is considered to be the product of climatic rather than cultural changes.

The argument about human involvement in recent vegetational changes is never easy to resolve. The presence of cereal pollen is often taken as evidence of human settlement and cultivation and therefore human potential for habitat modification. Cereal type pollen grains (40–50 μm) were found in the Rajasthan desert as far back as 9,300 b.p. and V. Mittre (Lucknow) reported even earlier finds of large Gramineae pollen in India. The Middle East records of cereal pollen were collated by A. Leroi Gourham (University of Paris); in Iran cereal pollen has been found in deposits going as far back as 60,000 b p., thus demonstrating the existence of, presumably, the wild progenitors throughout the last glacial climatic episode. In Lebanon and Syria, herbaceous pollen was low during the forested times of the last glacial, but increased with forest decline (here cereal pollen is defined as being in excess of 60 μm).

The recognition of true cereal pollen, however, is not a matter over which all palynologists agree. Size criteria are most frequently used, but it is well known that mounting media can alter the sizes of pollen grains, they may swell in the course of time, and also some wild grasses have large pollen grains. E. Kohler and E. Lange presented some preliminary data on scanning electron microscope studies of grass pollen in an attempt to define cereals on more reliable morphological grounds. Only four genera (12 spp.) have been examined in detail so far, but four distinct types of sculpture have emerged. Sculpture alone however, does not provide a means of separating cereal from wild grass pollen. It is hoped that combinations of size, pore and sculpture type criteria will permit a more critical definition of pollen types within the Gramineae. Such a definition is vital to the furtherance of the study of primitive agriculture, its extents and its influence upon vegetation in the Middle East and India. ☐

review article

Modelling the structures of amorphous metals and alloys

J. L. Finney*

The application of random packing models to amorphous metals and metal–metalloid alloys is discussed, with particular reference to the effects of soft potentials, boundary conditions, and density. The local atomic organisations found leads to structural speculation concerning the glass transition, and the existence of 'amorphous polymorphs'.

SINCE the late 1950s when Duwez developed his 'splat-quenching' technique for the rapid cooling of liquids[1], a range of non-equilibrium, solid metallic alloy phases have been produced. Many such materials, and others produced by sputtering, and by electro-, chemical, and vapour deposition, exhibit interesting and potentially useful mechanical, magnetic and corrosion-resistant properties More recently, amorphous (pure) metals have been prepared by vapour deposition on cold substrates[2–4].

Many (though by no means all) of these materials are non-crystalline[5]. This non-crystallinity raises severe problems when we try to understand their structure–property relations. The absence of a regular underlying lattice organisation makes it difficult (and often dangerous) to apply many of the powerful methods of solid-state physics to such systems. Their structural characterisation is related more to dense liquids than crystalline solids. Rapidly quenched liquids at least might be directly related to the liquid organisation from which the 'glassy' solid is formed; thus there is little reason even to expect crystal nucleation to have taken place.

Use of models

This conceptual and mathematical vacuum—the absence of mathematical formalisms capable of dealing adequately with dense, non-crystalline aggregates of atoms or molecules—has been partly filled by the development of methods of structural modelling. Models of particular non-crystalline atomic or molecular aggregates can be built both in the laboratory[6–14], and on a computer[15–28]. Properties predicted by such (numerical) models can be compared with those observed experimentally, and disagreements used to improve the original model. Such modelling techniques were used extensively in the early days of crystallography. The question was asked: 'what structural arrangement of molecules can I construct which is (1) consistent with what I know about the way these molecules interact, and (2) consistent with the existence of a regular, repeating lattice in three dimensions?' Thus, the crystal chemist explained the structures of the rare gas solids in terms of close-packing of hard spheres, or ionic structures in terms of charge and size effects, and of diamond in terms of tetrahedral covalent bonding. Such explanations began as 'ideal' structures, which were later improved by taking into account more subtle effects such as the detailed nature of the potential function, polarisation and three-body interactions

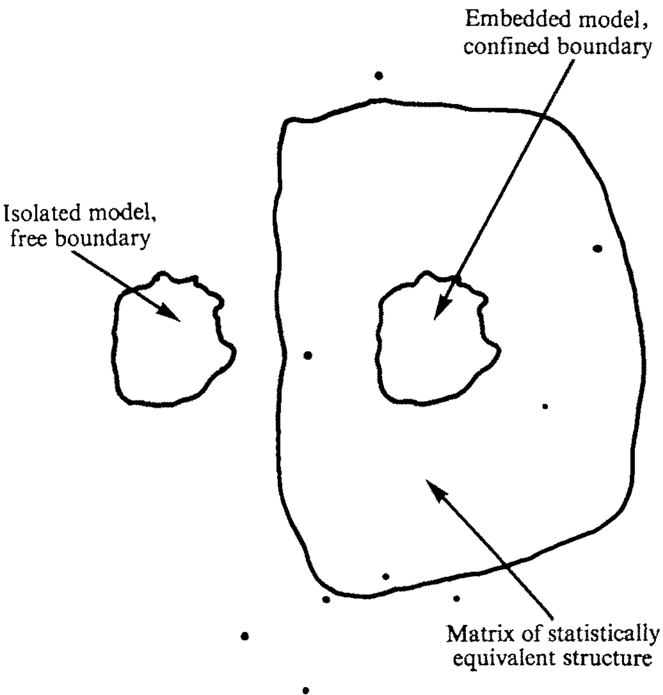
Modelling techniques as applied to non-crystalline solids are in principle similar. We ask a slightly different question: 'What structures can I build which are (1) consistent with the interactions between molecules (the 'potential function') but (2) not constrained by a regular lattice? As in the crystalline case, we establish first an 'idealised' structure of our amorphous metal or alloy. This idealised structure should relate to the real structure as an idealised crystal structure (such as a cubic close-packing of hard spheres) relates to the real crystal. The idealised structure is then improved upon to explain in more detail the structure and properties of the real non-crystalline material.

Such modelling techniques have been applied specifically to a variety of non-crystalline solids, for example, silicate and other oxide glasses[6–8], amorphous Si and Ge (refs 10, 26, 27), amorphous ice[29], and chalcogenide glasses[30–32]. Although different systems present different problems to the modeller, the principles—and the pitfalls—are essentially similar in all cases. The structures of amorphous metal films (for example, Co, Ni (refs 2, 4) and glassy transition metal–metalloid alloys (NiP (ref. 5), CoP (ref. 28)) have been explored extensively by modelling and illustrate the principles and problems particularly well

Specific application

To answer the model-builder's question above, we need first to strip the potential function down to those aspects which we think are structure-determining, and second to devise a method of

Fig. 1 The embedding problem. The structure of our finite model must correspond to that structure which would be consistent with its embedding in a matrix of (statistically) equivalent structure.


Embedded model, confined boundary
Isolated model, free boundary
Matrix of statistically equivalent structure

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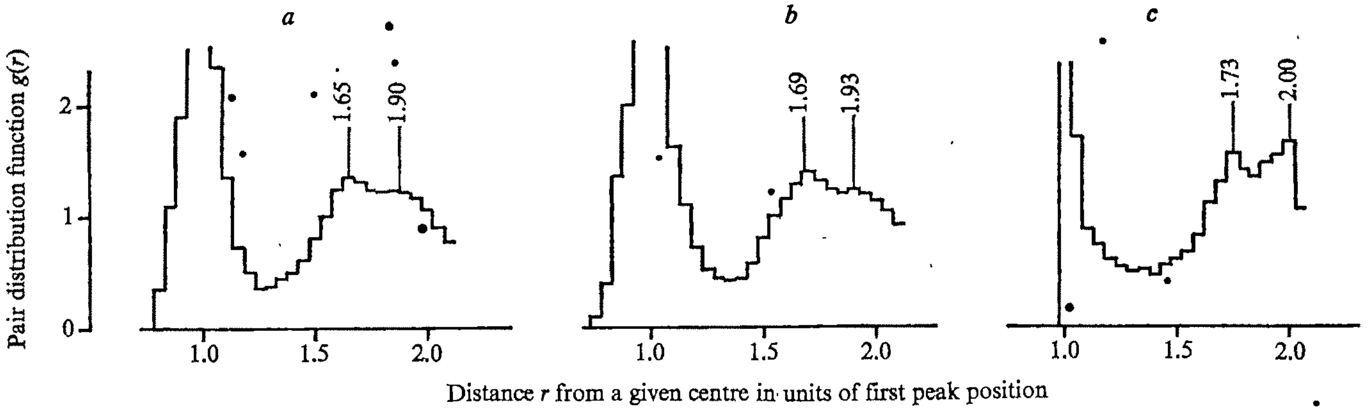

Fig. 2 Pair distribution functions of *a*, an amorphous bulk $Ni_{76}P_{24}$ alloy[5], *b*, vapour-deposited amorphous Co (ref. 2), *c*, a random close-packed assembly of hard spheres[14]. $g(r)$ is the probability of finding an atom at distance r from any atom. The packing density of (*c*)—the ratio of total volume occupied to the total volume of spheres—is 0.6366 ± 0.0004 (ref. 14). The relative positions (r_2/r_1 and r_3/r_1) of the two components of the split-second peak are indicated.

building an array of a large number of such simplified molecules, consistent with what we otherwise know of the real system. In the metallic case, we simplify our atoms initially to hard spheres, expecting the structure to be determined largely by the repulsive core of the real potential function, as is known to be the case in crystals and liquids[12,33-35]. However, when we try to put our idealised atoms together into a non-crystalline dense assembly, we run into difficulties which we must examine.

Boundary problems

Consider for example a hypothetical bulk amorphous metal. Any model we build can represent no more than a small sample of the bulk material However, the model must be capable of extension indefinitely in three dimensions, without any significant change in structure. This gives rise to what might be called the 'embedding problem' (Fig 1). An adequate model must be consistent with its infinite extension, the model boundary must therefore be consistent with the constraints that would be imposed by the presence of similar arrangements of molecules at that boundary. This is particularly relevant when we consider the variety of methods used to prepare amorphous metallic assemblies. The growth process and the boundary conditions operative during the sequential deposition from the vapour of a thin film of amorphous cobalt are very different from those relevant to the production of an amorphous alloy by rapid quenching of the liquid *A priori* there is no reason to assume identical structures will occur in these two extreme cases. A multiplicity of different amorphous structures may well exist, depending on the material and how it is prepared. Our modelling procedures should be capable of handling these possibilities

Experimental comparisons

A general problem of characterising the structures of non-crystalline materials concerns the relatively low information content of the usual structural measurements. X-ray and neutron scattering from crystals can give a relatively vast amount of very specific spatial and dynamic information. In non-crystalline materials the spatial information obtainable is limited to a statistical average projection of the structure on to one dimension. By carefully Fourier transforming the coherent scattered radiation we can obtain a pair distribution function (PDF) (see Fig. 3). This tells us the probability of finding an atom at a distance *r* from any given atom. It is the crystallographer's Patterson function projected on to one dimension. This PDF is a relatively insensitive structural measure but because it is easily accessible from both real materials and model coordinates, agreement of the model and experimental PDFs is an essential filter for postulated models. Agreement is a necessary, but far from sufficient condition which a successful model must fulfil. Because of the series termination problems inherent in the Fourier transformation of the scattering function[36], it is often preferable to compare real scattering functions with those predicted by a given model, especially when considering refined models. However, the structural reasons for disagreements between model and experimental scattering functions are not obvious in the scattering functions themselves. Thus, in what follows, we will use the PDF filter to examine postulated models and refinements, together with the densities of model and real material This somewhat old-fashioned property is often easily measured, and provides an additional powerful filter which leads to rejection of many postulated models The relatively high densities of amorphous metals and alloys are, in fact, not easily reproduced in model structures, because of the imperfectly understood packing restrictions in non-crystalline dense assemblies

Early models

The first model to be widely applied to amorphous metals and alloys was constructed in the laboratory by packing together as densely as possible a large number of equal steel spheres, taking precautions to prevent ordering effect at the boundaries[14]. This work was initiated by Bernal in the late 1950s (refs 12, 13) to try to elucidate the geometrical nature of the liquid state. Similar work was performed independently by Scott in Toronto[14] Cohen and Turnbull suggested[37] such models might be applicable to the then still hypothetical amorphous metal. However, its first specific application was to electrodeposited $Ni_{76}P_{24}$, whose X-ray scattering could not be reconciled with any realistic model based upon microcrystalline units[5].

Fig 2 compares the PDFs of electrodeposited $Ni_{76}P_{24}$, vapour-deposited amorphous Co (refs 2, 3), and the model dense hard-sphere packing out to two sphere diameters Qualitatively, the agreement of both real systems with the model is good The second peak is split in all three cases; at the time the hard-sphere model alone was able to account for this peak splitting. Moreover, the densities of model and experiment are of the same order, although precise quantitative comparisons are difficult as there is a degree of freedom in scaling the model to the real assemblies

However, the quantitative agreement is far from perfect. The relative intensities of the two components of the split-second peak are predicted the wrong way round, and their relative positions r_2/r_1 and r_3/r_1 (measured with respect to the position of the 1st peak r_1) are slightly discrepant (Fig. 2). Moreover, how justified are we in applying such a simple single component model to both (1) a binary alloy stable in the bulk, and containing a slightly smaller atom of different chemical nature (the 'glass former' phosphorus), and (2) a thin film of material containing only one metallic component, and produced by sequential vapour deposition? We cannot really ignore the effect of a glass former, which may induce specific local structuring effects in bulk NiP case, even though the effect on the PDF may be small. Nor should we necessarily expect the film material, prepared by sequential deposition from vapour, to have a structure similar to that of a model prepared to simulate specifically a bulk system.

Nevertheless, this agreement of PDF and density was sufficiently good to encourage proceeding on the postulate that the idealised hard sphere model incorporated the essential geometry of both types of system. We can now consider possible improvements to this idealised structure by examining the effects on it of, for example, different building methods, binary and more complex mixtures, different potential functions, and variations in boundary conditions. We are thus able to relate the above idealised structure more closely to that of the real materials.

Model refinements and alternative structures

Building and measuring large physical models in the laboratory is a painstaking business, and involves several imperfectly-controllable variables. Transferring the model-building to a computer facilitates tighter control of these variables, and enables structural variations to be investigated relatively rapidly. Nevertheless, there are severe restrictions on computer modelling. For example, it is very difficult to program those collective rearrangements of molecules that are necessary in order to reproduce the high densities obtained in laboratory models and the real materials, especially if large models are required. On the other hand, subtle structural effects of different potential functions can be readily examined, as can the effects of boundaries. Molecular dynamics simulations can generate structures consistent with external thermodynamic constraints in a way that both 'static' laboratory and computer building cannot hope to do. Even so, the long relaxation times of non-crystalline or glassy materials, compared with the elementary molecular dynamics time step, presently limit full simulation calculations to quenching rates much higher than those achieved in the laboratory; hence these methods can produce little more than configurationally arrested liquids. However, taking together these three building methods, we can tackle effectively many of the interesting static and dynamic problems of glasses and non-crystalline solids.

Laboratory models

Little further laboratory modelling of amorphous metals and alloys has been reported. A recent study of 60:40 binary mixtures of hard spheres, size ratio 4:5, by Scott and Koniuk (personal communication) does show a reversal in the split-peak intensities, and also a reduction of the r_2/r_1 ratio to 1.89, much closer to the real $Ni_{76}P_{24}$ value. The r_2/r_1 ratio, however, remains unchanged at about 1.73.

Computer algorithms

Various procedures have been used to build hard-sphere packings both in the bulk and as thin films. The most widely used is the sequential building method of Bennett[15], where each sphere is added in turn to a previously-existing tetrahedral site. The choice of site can be varied. Bennett originally used both 'global', and 'local' criteria. In the former scheme, a sphere is added at that site closest to the centre of the existing cluster, while the 'local' criterion chooses that tetrahedral site nearest to the plane of the three

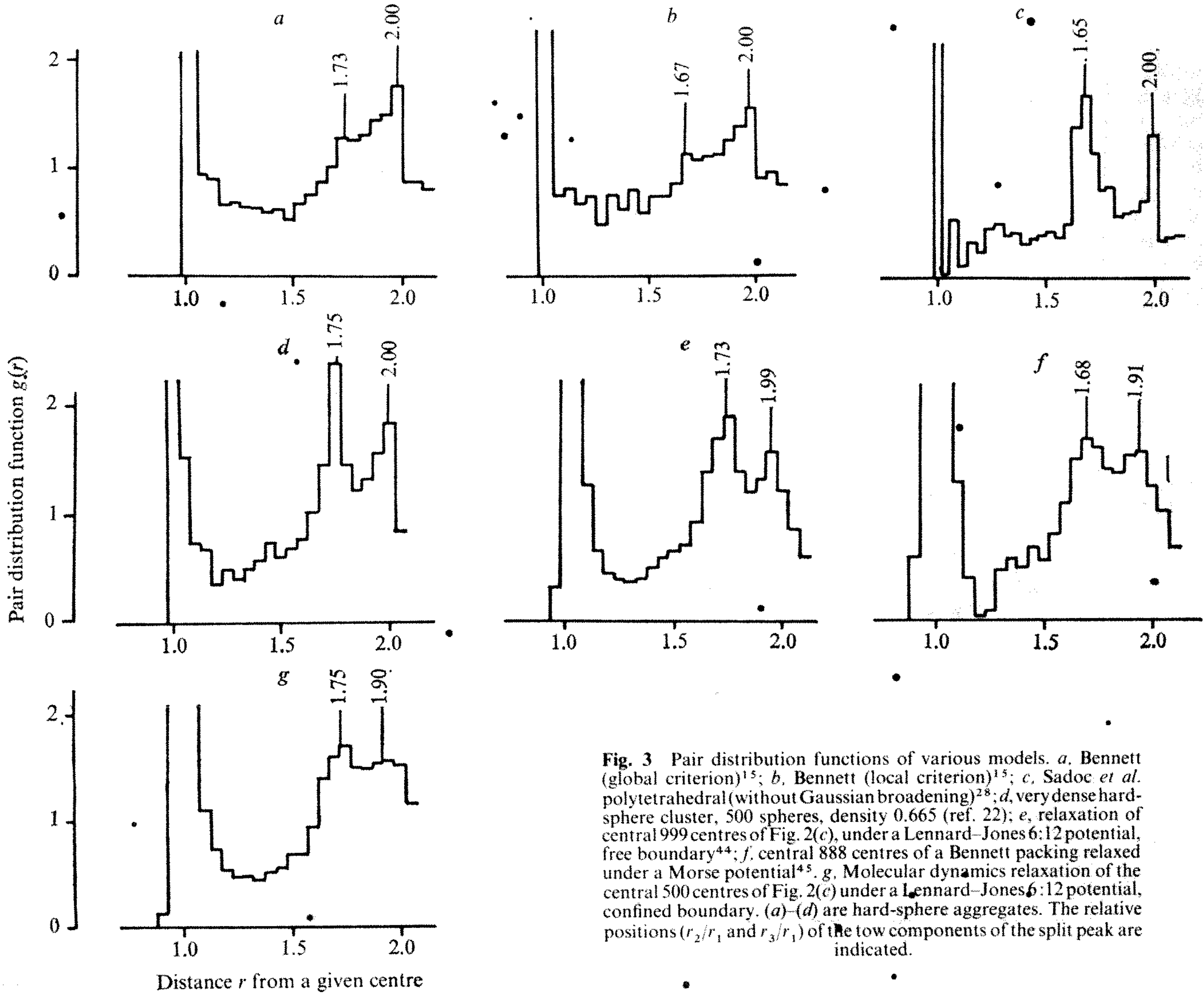

Fig. 3 Pair distribution functions of various models. *a*, Bennett (global criterion)[15]; *b*, Bennett (local criterion)[15]; *c*, Sadoc *et al.* polytetrahedral (without Gaussian broadening)[28]; *d*, very dense hard-sphere cluster, 500 spheres, density 0.665 (ref. 22); *e*, relaxation of central 999 centres of Fig. 2(*c*), under a Lennard-Jones 6:12 potential, free boundary[44]; *f*, central 888 centres of a Bennett packing relaxed under a Morse potential[45]. *g*, Molecular dynamics relaxation of the central 500 centres of Fig. 2(*c*) under a Lennard-Jones 6:12 potential, confined boundary. (*a*)–(*d*) are hard-sphere aggregates. The relative positions (r_2/r_1 and r_3/r_1) of the tow components of the split peak are indicated.

spheres defining it. Clusters of up to 4,000 spheres were built, and densities, extrapolated to infinite volume to eliminate size and boundary effects, were significantly lower (about 4%) than achieved in the laboratory packings. Sample PDFs are shown in Fig. 3(*a* and *b*). In both cases, the peak splitting is incomplete, reflecting the lower model densities. The peak positions of the 'global' model remain as in the laboratory packing, while for the 'local' model, r_2/r_1 alone is at 1.67 in better experimental agreement.

The Bennett procedure is very simple and fast, and has been used extensively with different boundary constraints, and with varying criteria for sphere additions[15–21,25]. Several of these models reproduce r_2/r_1 relatively well, but fail to bring r_3/r_1, closer to experimental values. Moreover, and more seriously, the densities of such models are low. It seems that for hard spheres at least, reproducing the positions and relative intensities of the experimental split second peak components requires a model whose density is too low. The packing constraints in a sufficiently dense model seem to be inconsistent with the adequate presence of those local geometrical structures which are consistent with the experimental split-peak component intensities and positions. This density–local structure problem is illustrated further by the polytetrahedral model of Sadoc *et al.*[28]. The algorithm used tries to reproduce the structural effects of the glass-former phosphorus in an amorphous Co–P alloy. In crystalline Co_3P each phosphorus is surrounded by 9 cobalt atoms in an incomplete icosahedral organisation. Sadoc's algorithm attempts to reproduce this icosahedral organisation, albeit slightly distorted from ideality. In Sadoc's resulting 'polytetrahedral' model, the relative peak heights are reversed, and the r_2/r_1 ratio is brought down to 1.65; r_3/r_1 remains at 2.00 (Fig. 3*c*).

However, this model also is not densely packed, having an average density 18% lower than random close packing[38]. It can be considered as an aggregate of dense, distorted icosahedral units, with considerable void space between them[38]. Thus, although the PDF of this inhomogeneous, clustered polytetrahedral model is qualitatively better than the homogeneous hard-sphere model, a consequence of forcing this clustering is, through the packing constraints, to prevent the achievement of a sufficiently dense assembly.

As long as we use hard spheres, the local structure which can explain the split second peak seems inconsistent with the high densities obtained in real materials. This is further underlined by an entirely different construction algorithm which simulates the compression of a hard sphere gas[22,24]. Here, collective rearrangements can be (rather laboriously) programmed, and very high densities achieved provided a free boundary is maintained. Figure 3(*d*) shows the PDF of such a 500-sphere cluster of packing density 0.664 (ref. 22). This is 4.2% higher than the 0.637 maximum obtained in the laboratory, a density which is thought to be the upper limit for a bulk random packing[14,39–41]. Here again, although we can reverse the split-peak intensities, we cannot budge their position[5]. From an examination of the nature of local clusterings, an interesting result arises. As density increases, the proportion of distorted icosahedral clusters—Sadoc's polytetrahedral clusters—increases slowly, although constituting barely a few per cent of the volume of the array[22]. Above a density of about 0.63, however, these 'spherically-polytetrahedral' units are forced out, demonstrating further that such locally dense clusters are indeed inconsistent with an overall high density.

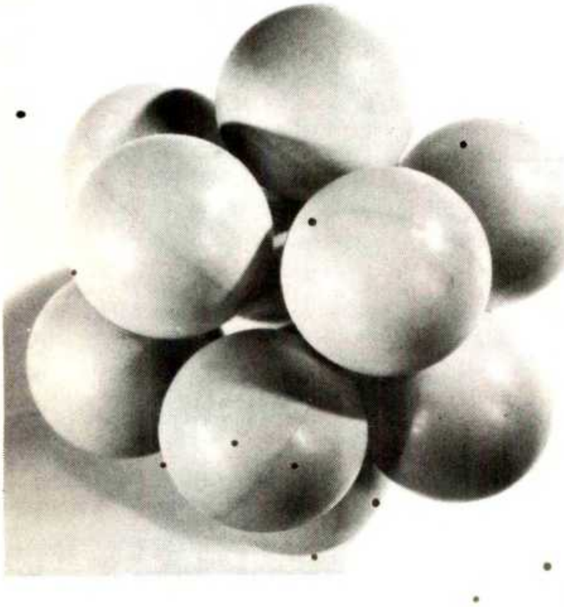

Fig. 4 Icosahedral arrangement of 13 spheres, related directly to the local geometry in 'spherically-polytetrahedral' models. This arrangement is that of minimum potential energy for soft spheres with a free boundary.

This result is not surprising for hard spheres. The geometry of the icosahedron is such that the 12 surrounding spheres have some freedom of movement on the surface of the central one (Fig. 4). For soft spheres—more like the real molecules—the 'squashiness' of the atoms would be expected to stabilise considerably such local units (Hoare and McInness, personal communication). The question thus arises as to the interplay of increased icosahedral stability at the local level and the overall packing constraints in a real dense structure. Are the two tendencies more mutually compatible when the artificial infinitely–hard repulsive core is softened?

Computer refinements and molecular dynamics

The effects of softer potentials have been examined by (1) relaxing laboratory or computer-built hard-sphere models under various soft potentials[43–46], and (2) very rapidly quenching liquid configurations by molecular dynamics[41,47–50]. The results from both approaches are in excellent agreement, and give good second-order models directly related to the first-order idealised hard-sphere model. However, various questions about local geometry, and their relevance to the glass transition in metallic systems, remain to be answered fully[38,51]. In one such relaxation calculation, the central 1,000 sphere coordinates in the laboratory-built hard-sphere model (Fig. 2*c*) were scaled to a Lennard Jones 6:12 potential and allowed to relax towards a potential energy minimum, in free boundary conditions[44]. Movements were generally small, being limited by the high density of the starting configuration. Fig. 3*e* shows the relaxed PDF. the split-peak intensities again reversed, and only a small inward movement of r_3/r_1 was observed. r_2/r_1 was unchanged, and it was suggested from geometrical arguments that this peak position should be sensitive to the softness of the repulsive potential. The degree of local distorted icosahedral clustering increased, but only slightly. These results are in close agreement with recent simulated Lennard–Jones glasses produced by almost instantaneous liquid quenching[41,48,49].

Slightly different results were obtained in a series of molecular dynamics calculations with confined irregular boundaries using the same potential and starting configuration[46]. Again an intensity reversal in the PDF was observed (Fig. 3*g*), and the inward movement of r_3/r_1 under the much higher pressure was significant. However, the degree of local 'spherical polytetrahedrality' was very low, such clusters having been presumably squeezed out under the very high pressure. Similar PDFs were obtained using a simulated harmonic potential[23]; the local geometry was not examined.

The Lennard–Jones 6:12 potential is thought to have too hard a repulsive core for realistic metallic atoms. Hence a relaxation of a hard-sphere packing under a soft Morse potential (unfortunately again in free boundary conditions) is of particular interest[45]. The PDF peak intensities reverse, and both r_3/r_1 and r_2/r_1 fall to values closer to the experimental (Fig. 3*f*). Thus, the position of the first component of the split peak does indeed seem sensitive to the softness of the potential. Such a relaxed model gives relatively good PDF and density agreement for amorphous metallic films. The agreement with the binary metal-metalloid alloys is less good. The relation of the r_2/r_1 shift to the local cluster arrangement is as yet uninvestigated in such very soft sphere assemblies.

Conclusions

The work discussed above is, because of the widely varying conditions, and assumptions made, difficult to assemble into a coherent and water-tight picture[52,53]. We can, however, make several generalisations about random packing, its application to real non-crystalline metallic alloys, and the problems and future prospects for modelling amorphous solids in general. We can also speculate a little about structural aspects of the glass transition and crystal nucleation.

We now have a much better idea of the nature of random packing. For hard spheres, a maximum packing density of about 0.637, is now well established for both modelling[14,39] and molecular dynamics extrapolations[40,41]. This maximum density can be achieved only by a relatively homogeneous assembly, with very little clustering into local icosahedral structures. Such clustering is prevented by the overall packing constraints. The homogeneous model is, however, polytetrahedral in that 70% of the assembly consists of relatively perfect tetrahedra[13]—naturally the densest local arrangement possible for four spheres. These tetrahedra are organised, however, not in the spherical clusters related to the stable icosahedral arrangements (Fig. 4) found in isolated atomic clusters[38,42,51,54], but in the twisted spirals of Bernal's 'pseudonuclei' (Fig. 5)[13].

In adequately dense packings of slightly soft spheres, the tendency towards local 'spherically polytetrahedral' clusters (Fig. 4) increases, but only slightly[44]. This increase is certainly very much less than that suggested in the much less dense specifically polytetrahedral models. 'Spiral-polytetrahedrality' (Fig. 5) is more stable under the local packing constraints found in dense assemblies. As long as the overall density remains high, there is insufficient volume available to allow the formation of many disorted icosahedra and their associated inter-cluster voids. It would seem that for molecules such as the rare gases where a Lennard–Jones 6:12 potential may be reasonable, the glass transition might more realistically be associated with the formation of relatively stable 'spirally polytetrahedral' clusters[13] than the 'spherically polytetrahedral' ones stable in isolation[38,42,51].

For even softer potentials, the situation may be different. The extra freedom conferred by the softness of the potential may change the relative stability within a dense packing of spherical against spiral tetrahedral assemblies. A detailed geometrical analysis of such a relaxed assembly would help clarify this point.

The hard sphere packing provides an idealised model of the structure of several amorphous metal films and metal–metalloid alloys. All modifications made to achieve a better model result in improved intensities of the split second peak of the PDF (Fig. 3)[22,23,43–45]. Only very soft potentials improve the position of the first component of the split peak (Fig. 3g)[45]. The relaxed hard-sphere model is indistinguishable from a Lennard–Jones glass[48], while the hard-sphere model itself is indistinguishable from a hard-sphere glass as produced by molecular dynamics[41].

The structural effects of boundary conditions can be significant[38,44,46]. Thus we should not necessarily expect vapour-deposited materials to have the same structure as those produced by liquid quenching. Moreover, a splat-quenched structure and vapour-deposited structure may well not be mutually accessible directly. This underlines the possibility of 'amorphous polymorphs' of the same substance, and the possibility of different amorphous structure for different substances[38]. Thus it would be interesting if amorphous phases of the same material could be produced by different routes, and their structural differences examined in detail, together with the possible routes for transformation between them[51,52]. The clustering effects of glass-formers are likely to be significant though they are not well worked out.

Future modelling work on amorphous assemblies of spherical atoms must take adequate account of both the local effects of the potential functions and the overall packing constraints as reflected in the density and the boundary conditions. Structure can be very sensitive to both these factors. As a filter of possible model structures, agreement with the PDF is a necessary though far from sufficient condition. More attention should be paid to the old-fashioned property of density—qualitative agreement with PDF is hardly convincing if the density is unrealistically low.

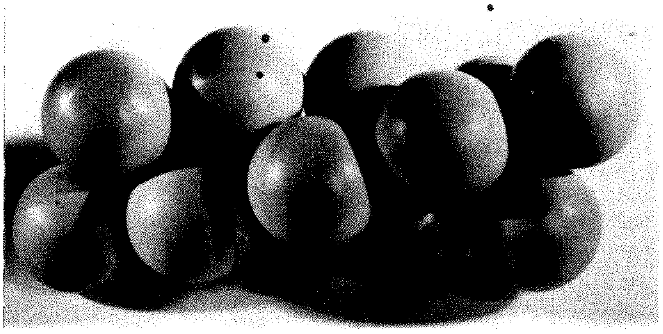

Fig. 5 A 'spirally-polytetrahedral' arrangement of 13 spheres, the so-called Boerdijk spiral of face-sharing tetrahedra. Such arrangements occur frequently in random packings of hard spheres.

The necessity to achieve high density models—and structural consistency with a bulk structure—raises severe difficulties for sequential building methods. These are basically unable to account for the high densities of real amorphous metals and alloys. Full molecular dynamics calculations can help overcome this, although the method is restricted severely by real time limitations. Although it is relatively straightforward to simulate a very rapidly-quenched liquid, the computer time required to simulate a quench on a realistic laboratory time scale, or a vapour deposition, is presently prohibitive. Thus, the detailed structural nature of the glass transition is not directly accessible thereby. Progress might be made by combining molecular dynamics with energy minimisation techniques[52], as has been done for the much more difficult but related protein folding problem[55,56]. These comments apply in general to the modelling of other glassy systems. Modelling is, however, still an inadequate substitute for more analytical approaches to the problems of local correlations in very dense, disordered systems. Although the last fifteen years of modelling a variety of amorphous solids, from the metallic systems considered here to oxide and chalcogenide glasses, has drastically increased our understanding of various non-crystalline systems, the methods become rather cumbersome when looking at refinements of first-order models. Moreover, especially in chalcogenide and more complex metallic alloy systems, the constraints that should be applied to the model-building may be insufficiently well formulated for the resulting models to be realistic. The interplay between the predicted properties of a postulated model, comparison with the real material, and feed-back to improve the model can be exceedingly time-consuming, and perhaps inconclusive once the basic idealised structure has been established. As the real amorphous systems we are interested in become more complex, the more difficult it becomes to handle, by simple modelling, the possible variables and possible heterogeneity of the structure. In some ways it is too easy to build models. To do it realistically, to represent a bulk system with adequate density, PDF, and density fluctuations in ill-defined or complex mixed systems is very difficult. And rather than turning a handle *ad infinitum* to improve marginally our models of simple systems whose basic structure we understand, we should pay more attention to the absence of an underlying theoretical framework, an absence which made modelling necessary in the first place. Only when such an adequate analytical approach to very dense, non-crystalline systems has been established—without the problems attached to integral equation approaches to the liquid state—will a full understanding of the complexities of these amorphous structures, their dynamics, their glass transitions and nucleation process, be realised. Data from models will have a role in the development of such techniques[57].

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articles

New palaeomagnetic evidence fails to support rotation of western Newfoundland

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Palaeomagnetic results from Ordovician to late Precambrian rocks in western Newfoundland are inconsistent with Wegener's hypothesis that the island has rotated 30° anticlockwise. Previous evidence apparently supporting this hypothesis is shown to be inconclusive.

WEGENER[1] proposed that Newfoundland has rotated 30° anticlockwise away from Labrador, implying a nearby pivot. A palaeomagnetic test of this motion is, in principle, straightforward, provided that test sites are carefully chosen (Fig. 1). Even so, the resolution obtainable may be small or zero, being a function of both the rotation angle R and the angular distance θ between pivot and ancient pole. Figure 2 illustrates this for Newfoundland ($R=30°$), where values of P ('resolution') approaching 30° are obtainable at Lower Palaeozoic sites suitable for testing possible Devonian or later rotations. Hence a near-optimum test is feasible because of the low latitude of ancient Newfoundland.

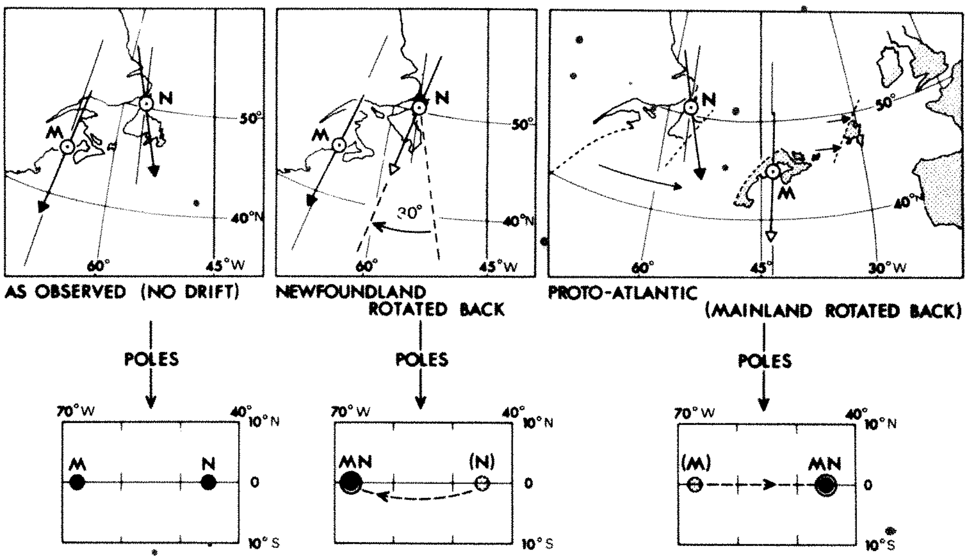
After an inconclusive application of this test[2,3], Black[4] compared Palaeozoic rocks of matching age in western Newfoundland and mainland eastern Canada, concluding that his data uphold a 30° anticlockwise rotation of at least western Newfoundland during the middle to late Devonian. Black argued that this would explain observed regional structures in the north-eastern Appalachians. Except for differences in latitude, his widely quoted Cambrian and Devonian comparisons conform strikingly to the positive test in Fig. 1. Regional and local tectonics[5,6] and seismic results from the Gulf of St Lawrence[7] have been cited as evidence against this rotation. Further, Black's data were found inconclusive[8], because (1) the Cambrian remanence directions inferred in Newfoundland and New Brunswick (Fig. 3, S, R) are not significantly different; (2) the respective "Devonian" directions may not be contemporaneous; (3) by demagnetising only in alternating fields (AF) to 300 Oe, he was unlikely to have distinguished different high-stability components. Also[9], since the mainland sites (Fig. 3, R) form part[10] of the Avalon tectono-stratigraphic zone, rocks on opposite sides of the Central Mobile Belt were being compared (R, S). Therefore data in Fig. 1 could equally be taken to support either a rotation of Newfoundland or a proto-Atlantic model[11].

New magnetic data

To meet these objections we undertook new magnetic studies of mid-Ordovician to latest Precambrian rocks in Newfoundland (Fig. 3, N1–N3, N5–N7), using standard equipment. The results, summarised below and in Table 1, will be reported in greater detail elsewhere. Comparisons are with published data from tectonically stable mainland regions (Table 2).

Useful palaeomagnetic results have been obtained from carbonate rocks[12–14]. We collected limestone of the Lower Ordovician[15,16] St George Formation at 10 sites (62 samples) in the Port-au-Port area (N1). The natural remanence (NRM), carried in different samples by magnetite, haematite or both (compare ref. 14), was weak and fewer than half of the samples could be demagnetised. Haematite samples were unaffected by AF treatment (800 Oe) while thermal treatment was inconclusive, but in the magnetite samples both methods produced comparable, better-grouped new remanence directions (Table 1). St George limestone from Cape Norman (N2) gave similar results. This magnetic

Fig. 1 Palaeomagnetic test of rotation (schematic). Declinations of hypothetical 'observed' remanence vectors (solid arrows) and corresponding pole positions are shown for Newfoundland (N) and the adjacent mainland (M). Hollow arrows depict the restored vectors when either Newfoundland is rotated back[1] or the mainland site is rotated to a presumed location before the closing of a proto-Atlantic ocean[11]. The pivots are at site N and 90°N, respectively. For convenience, poles have been located at the present equator. The two rotations here are indistinguishable[9]: an unambiguous test of the Newfoundland rotation requires comparison with a mainland site known not to have rotated. Stippled portions (right) correspond to the Atlantic faunal realm[11].



behaviour in Newfoundland limestones parallels that found earlier in the mid-Ordovician Trenton limestone[12] (M1), where it was shown that the stable component originated before the completion of diagenesis. This suggests a similar origin in the Port-au-Port limestone, but proof is difficult and it is uncertain whether one should correct for the gentle (12–15°) tilt which is associated[10] with the Taconic (pre-mid-Ordovician) or Acadian (Devonian) orogeny or both. The (uncorrected) mean directions for areas N1 and N2 after AF or thermal treatment are all similar (Table 1). Pole N1 (Table 2) is based on sample rather than site statistics, since too few samples per site remained measurable after AF treatment. The tilt-corrected pole is 22°N, 120°E. Pole N1 is not significantly different from Pole N4 (ref. 13) for the St George Formation, where no tilt correction was reported.

A result[17] from pillow basalt and sheeted dikes of the Moreton's Harbour Group (N3) is useful for comparison with the St George Formation, since different rock types of similar ages are being compared. Most or all of the 8-km thick Moreton's Harbour sequence has been attributed[18,19] to island arc volcanism in the Lower[19] to Middle[18] Ordovician. Although these rocks lie in the Central Mobile Belt (Fig. 3), it seemed reasonable to include them in a test based on western Newfoundland, since they probably originated as part of the North American margin (D. F. Strong, personal communication). A stable remanence in magnetite, isolated by AF demagnetisation, presumably was acquired not later than the generally low-grade greenschist metamorphism of these rocks. The tilt-corrected pole (Table 2) is not significantly different from poles N1 and N4 (Fig. 4).

Lower Cambrian[15] Bradore sandstones sampled by Black[4] at St John Bay (Fig. 3, S) were re-sampled more extensively[20] at Canada Bay (N5). The NRM, carried by haematite, had a mean direction as in Black's AF-treated samples (Table 1), but by thermal demagnetisation a stable component misaligned with both the NRM and Black's direction was isolated at 665 °C: this shows that the objection[8] to exclusive low-AF treatment is well-founded. This component probably pre-dates the gentle (15–25°) Taconic and/or Acadian tilt of the sediments, though a post-tilting origin of the entire NRM is not impossible[20]. The stable direction corresponds to a reverse pole at 23°N, 160°E

Table 1 New palaeomagnetic results from Newfoundland

Area no.	Rock unit/area	Geological age	*N*	(*n*)*	Mean NRM direction† Declination (°)	Inclination (°)	*k*	Treatment‡	Mean direction after treatment† Declination (°)	Inclination (°)	*k*	α_{95}(°)
N1	St George limestone, Port-au-Port area	Early Ordovician[15]	5S§	(14s)	179.5	+62	63	300 Oe	181.5	+25	910	2.5
			15s§	—	179.5	+57.5	13	300 Oe	182	+23	90	4
			6s	—	159	+61.5	49	500 °C	183	+18.5	200	5
N2	St George limestone, Cape Norman area	Early Ordovician[15,16]	5s	—	164.5	+73	70	400 °C, ‖ 300 Oe ‖	177	+22.5	120	7
N3	Moreton's Harbour basalt	Early to mid-Ordovician[18,19]	11S	(56s)¶	114	+66	8	150 Oe	(116.5 176	+57 +16	9 9	16.5) 16
N5	Bradore sandstone, Canada Bay	Early Cambrian[15]	9S	(54s)	153.5	+52	13	665 °C	146.5	+18	110	5
N6	Bradore sandstone, St John Bay	Early Cambrian[15]	2S	(13s)	198	+66	—	665 °C	132.5	+25.5	—	—
S	Bradore sandstone, (Black's result)[4]	Early Cambrian[15]	10s	—	?	?	?	300 Oe	151	+44.5	27	9.5
N7	Cloud Mountain basalt	Latest Precambrian**	3F	(10S)	159.5	+73	14	100 Oe	(142 127	+50 +36.5	840 1250	4.5°) 3.5°

*N, number of flows (F), sites (S) or samples (s) used in averaging; (*n*) number of sites or samples studied.

†Angles rounded to 0.5°; *k* values greater than 100 are rounded to nearest 10; where two sets of data are given, those in brackets were obtained before correction for geological tilt and those without brackets are tilt-corrected and were used in Table 2. In all other cases, no tilt correction was made.

‡Stepwise thermal or AF demagnetisation, with fields quoted in peak oersteds.

§Site and sample statistics for the same samples are shown for comparison; samples (second row) are used in Table 2.

‖Three samples were treated to 400 °C, two samples to 300 Oe.

¶Reduced to 46 samples (11 sites) after treatment.

**^{40}Ar/^{39}Ar date of 605±10 Myr cited for Long Range dikes (ref. 25), which are considered (refs 24, 25) to be contemporaneous with Cloud Mountain basalts.

Table 2 Palaeomagnetic poles for Newfoundland and mainland North America

Area no.	Rock unit/area	Geological age*	*N*†	Treatment†	Pole position Latitude (°)	Pole position Longitude (°)	Pole position dp,dm (°)	Polarity†	Mean rotated pole‡ Latitude (°)	Mean rotated pole‡ Longitude (°)
N1	St George limestone, Port-au-Port area	Early Ordovician	15s	a	30 N	119 E	2,3	R	26 N	86 E
N3	Moreton's Harbour basalt	Early to mid-Ordovician	11S	a	32 N	130 E	9,17	R	27 N	96 E
N4	St George dolostone, Daniel's Harbour	Early Ordovician[15,13]	8S	a	29 N	125 E	3,7§	N	25 N	92 E
M1	Trenton, limestone, New York	Mid-Ordovician[12]	29s	a	36 N	114 E	3,5	R		
M2	Front Range intrusives, Colorado	'Cambro-Ordovician'[26]	18s	a	37 N	122 E	6,12	R		
			14s	t	44 N	100 E	5,11	R		
			—	a+t	40 N	111 E	—	R		
N5	Bradore sandstone, Canada Bay	Early Cambrian[15]	9S	t	23 N	160 E	3,5	R	30 N	128 E
N7	Cloud Mountain basalt	Latest Precambrian	3F	a	5 N	172 E	2,4	R	17 N	146 E
M3	Franklin intrusives, Northwest Territories	Latest Precambrian[29,31]	46S	a	6 N	166 E	4,4‖	M		
N8	Indian Head anorthosite	'Grenville'[32]	5S	a	8S	158 E	15,20	N	1 N	136 E
M4	Morin anorthosite, Quebec	'Grenville'[33]	13S	a	0	164 E	9,12	R		
M5	Northern Grenville anorthosite, Ontario	'Grenville'[34]	4S	a	8 N	161 E	6,6‖	R		
M6	Whitestone anorthosite, Ontario	'Grenville'[35]	8S	a	5 S	168 E	6,10	R		
M7	Charlevoix anorthosite, Quebec	'Grenville'[36]	5 S	a	7 S	148 E	12,16	R		

*Ages are discussed in the references (see also Table 1). Ref. 26: Radiometric ages in the range 601–427 Myr are cited. Ref. 29: The authors cite numerous K/Ar ages with 'best mean age' 650 Myr, but they adopt 675 Myr, to allow for argon loss. Ref. 31: Other Franklin igneous sites have K/Ar ages matching an isochron of 625 Myr. Refs 32–36: 'Grenville' ages in the range 0.9–1.0 × 10^9 yr are cited, based on radiometric data or on inferred time of magnetisation.

†*N*, see footnote to Table 1; Treatment: a, alternating-field; t, thermal; a+t, mean of poles based on a and t treatment; Polarity: N, normal; R, reverse; M, mixed.

‡Average values are quoted for 30° clockwise rotations about pivots A (51.5°N, 56.5°W) and B (48°N, 59.5°W) (Fig. 3).

§Recalculated from original data.

‖Error circles (α_{95}) instead of ovals are cited by authors.

(Table 2), or 29°N, 167°E after tilt correction. Bradore standstones from St John Bay (N6) gave comparable (virtual) poles.

The gently dipping Cloud Mountain plateau basalts (N7) were sampled at 10 sites. They have been correlated stratigraphically[21] and through magnetic similarities[22,23] with plateau basalts in south-east Labrador of presumed late Precambrian to early Cambrian age[21]. Strong and Williams[24] consider the Cloud Mountain basalts to be co-magmatic with and contemporaneous with the chemically similar Long Rang dikes, which have a ^{40}Ar/^{39}Ar dating of 605±10 Myr (ref. 25). Only three separate flows could be identified. AF treatment isolated a stable component carried by magnetite, resulting in greatly improved within- and between-flow vector groupings. Its mean direction after tilt correction corresponds to the pole shown in Table 2. We note that a residual secular variation component may be present in these few flows.

Rotation test

There is no unique pivot for the postulated rotation of western Newfoundland. Black[4] relocated his Newfoundland palaeomagnetic poles by rotating the island 30° clockwise separately about two extreme pivots A and B (Fig. 3) using the mean restored pole position to compare with poles on the mainland. A similar procedure is followed here (Table 2). The uncertainty in pole positions due to averaging about the two pivots is ⩽1°.

Out of the three identical Ordovician Newfoundland poles, N1 and N3 are in turn identical with, and pole N4 is close to, the Trenton limestone pole[12] (M1). Here 'identical' is defined non-rigorously by an overlap of the 95% confidence ovals (Fig. 4). Pole M2 from the Front Range Intrusives[26] of uncertain Cambrian to Ordovician age is shown without oval, being the average of two poles equally weighted in ref. 26, based on AF and thermal treatment, respectively; the former but not the latter pole is identical to poles N1, N3, N4 and M1. With Newfoundland rotated back, the 'N' and 'M' poles become discordant, except pole N3 for which the test is inconclusive. These results are

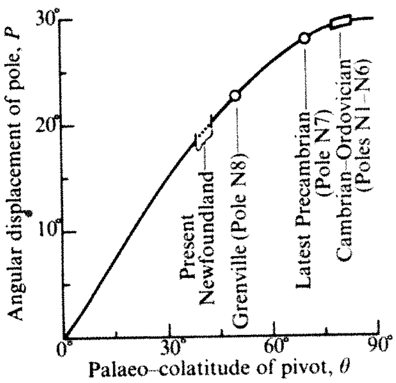


Fig. 2 Resolution (*P*) obtainable by palaeomagnetism for testing rotation (*R*) of a land mass, as a function of the angular distance (θ) between pivot and ancient pole, where[9]: $1-\cos P = \sin^2\theta\,(1-\cos R)$. *P* is the angular shift of the ancient pole on restoring the land mass to its pre-drift position. *P* cannot exceed *R*. Illustrated for the Newfoundland hypothesis ($R = 30°$) using actual results (Table 2). Note that, with minor error in θ, site locations N1–N8 may be substituted for the average pivot locations A and B (Fig. 3).

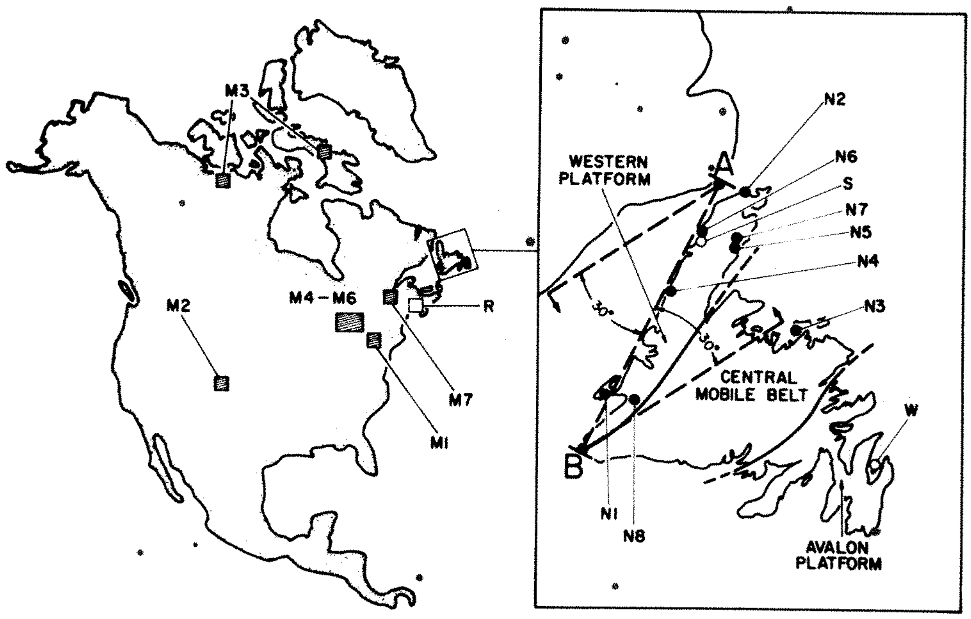


Fig. 3 Palaeomagnetic sites for testing rotation of Newfoundland (Tables 1, 2). R, Ratcliffe Brook, New Brunswick • (Lower Cambrian)[4]; M1–M7, other mainland sites. S, St John Bay[4]; W, Wabana[37]; N1–N7, other Newfoundland sites. A, B, alternative pivots[4] (Table 2).

incompatible with the proposed rotation, at least since mid-Ordovician time. A provisional Upper Ordovician pole (R. Van der Voo, personal communication) at 29°N, 118°E from thermally cleaned red sandstones of the Juniata Formation, Pennsylvania, is not significantly different from poles N1 (30°N, 119°E) N3, N4 and M1. This agreement may be due to insignificance of mid- to late-Ordovician polar motion relative to eastern North America, in which case the Juniata result is additional evidence against rotation of western Newfoundland.

Comparisons of the Bradore data[20] (N5) with those from Cambrian mainland localities that avoid the proto-Atlantic ambiguity are difficult, since the few reliable mainland poles (see ref. 27) have been cited as Upper rather than Lower Cambrian in age; also they are widely dispersed, suggesting rapid polar displacement. Hence a rotation test using the Bradore result seems premature. A Lower to Middle Cambrian pole at 13°N, 157°E for eastern Quebec was recently reported[28], however. Its fair agreement with the Bradore pole (23°N, 160°E) is destroyed when Newfoundland is rotated back. This may be taken as evidence against the proposed rotation.

On the mainland the youngest suitable Precambrian rocks are the Franklin intrusives[29–31]. Of numerous K–Ar datings, 675 Myr is the adopted age[30] cited for pole M3 (Table 2). but other Franklin diabases and lavas[31] give a pole close to M3 and are dated 625 Myr, compared with the 605-Myr age inferred for Cloud Mountain (N7). Figure 4 shows agreement between poles N7 and M3, but they strikingly diverge when Newfoundland is rotated back. Pending better knowledge of the Newfoundland–mainland age difference and of the time span averaged in the Cloud Mountain lavas, this result again is inconsistent with the rotation hypothesis.

The minimum radiometric age of 890–900 Myr of the Indian Head anorthosites[32] (N8) suggests that they may be contemporaneous with mainland rock formations dated at the time of the Grenville orogeny (1.0×10^9 yr). Pole N8 was compared with only mainland poles dated $0.9–1.0\times10^9$ yr, based on anorthosite (M4–M7)[33–36], to make it more probable that a synchronous Grenville event is being tested. Once again an initial agreement between 'N' and 'M' poles is lessened when Newfoundland is rotated back (Fig. 4). Unrotated, the oval of pole N8 overlaps all mainland ovals, whereas after rotation it misses two of these (M5, M6). The large size of oval N8, however, makes this test only marginally significant.

Significance of the test

Newfoundland data from the Ordovician, latest Precambrian, more marginally the Grenville and perhaps also the Cambrian, when compared with the mainland results, provide evidence against Wegener's[1] hypothesis. At the

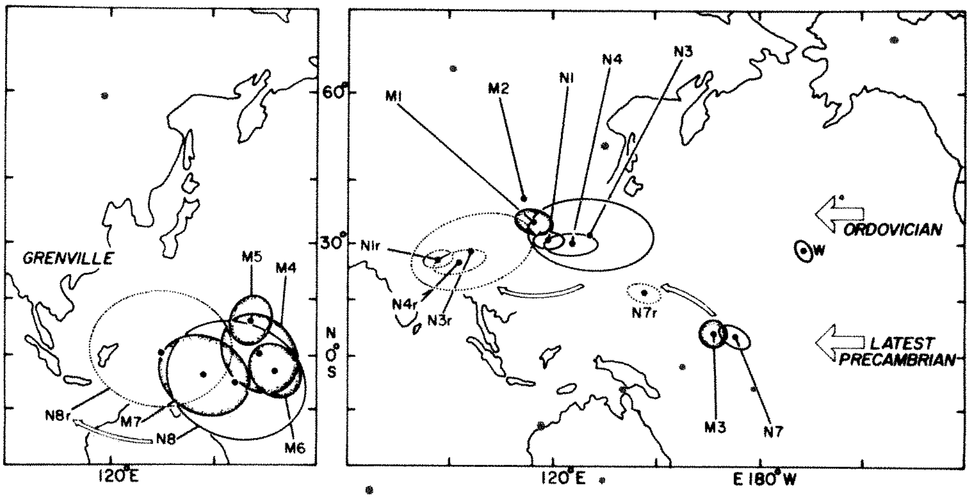


Fig. 4 Negative results of the rotation test for three age levels. Pole positions with 95% confidence ovals (Table 2) for the mainland (M) (shaded ovals), and Newfoundland (N) as observed (solid ovals) and after rotation (dotted ovals, site numbers followed by '*r*'). W, Wabana (Fig. 3 and text)[37]. Magnetic north or south poles are plotted to correspond to presumed north rotation poles.

same time we have verified that Black's[4] Cambrian results used by him in support of that hypothesis are inconclusive. Generally better data were available to us than to the earlier workers[2,4] in terms of statistics, magnetic control and comparative dating. Limitations are, however—(1) some large confidence ovals; (2) different populations (samples, sites, flows) being compared; (3) the small number of usable limestone samples (15) and of Cloud Mountain flows (three); (4) uncertainty regarding tilt corrections; (5) possible anachronisms between poles of any given age; and (6) the absence of a reliably Lower Cambrian mainland result to compare with pole N5.

Partly because of these limitations, we could not employ rigorous statistics. For example, an *F* test applied to negative evidence on rotation would ideally show, in each case with high probability, that the mainland and unrotated Newfoundland poles for a certain age are identical and that they become distinct when Newfoundland is rotated back. Comparing actual data, such as Ordovician poles N1, N4, M1, the latter condition is certainly met, but the former condition is only moderately probable. The difficulty is predictable from Fig. 4 and recurs in an *F* test of the Grenville poles. This restricts us to the use of confidence ovals for assessing significance.

We conclude that the evidence is inconsistent with western Newfoundland having rotated by 30° since the late Precambrian, and is consistent with the absence of any rotation, although a rotation by 5°–10° since the Ordovician is possible. To determine whether such lesser rotation actually took place calls for more refined palaeomagnetic data from Newfoundland and North America.

Finally, a Lower Ordovician pole from Wabana, eastern Newfoundland[37] (W, Figs 3, 4) is seen to be far displaced from poles N1 and N4 of similar age in western Newfoundland. This is compatible with the hypothesis[11] of a Lower Palaeozoic proto-Atlantic Ocean of undetermined width, in which the two portions of Newfoundland occupied opposite coasts. Poles W, N1 and N4 may be made to coincide by rotating Wabana towards Europe about some pivot in the central Atlantic.

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Current rifting episode in north Iceland

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A major rifting episode is now occurring in north Iceland. This started on 20 December 1975, with a basaltic eruption, an exceptionally intense earthquake swarm and movement on an 80-km segment of the plate boundary. Inflation and deflation of the Krafla caldera indicate upwelling of magma and injection into the rift zone. Historical records show that similar episodic rifting occurs in this region every 100–150 yr.

THE plate boundary between the American and European plates runs through Iceland as a zone of active volcanism, seismicity and graben structures. The two plates are drifting away from the boundary at a velocity of about 1 cm yr^{-1} (ref. 1), but the mechanism of this drifting process at the boundary itself has not been clearly observed until now. A spectacular rifting episode started in northern Iceland on 20 December 1975, and is still not completed in December 1976. Intense earthquakes, widespread ground deformation and magmatism are affecting an 80-km long segment of the plate boundary. We describe here the geological setting of these events, the main observations to date and an attempted interpretation.

In north Iceland the Neovolcanic Zone has a north–south direction. Towards the south it connects with the Eastern Volcanic Zone of south Iceland, but towards the north it is offset to the west by about 100 km, by the Tjörnes Fracture Zone, to join the Kolbeinsey Ridge (Fig. 1). The structure of the Neovolcanic Zone in north Iceland is dominated by large swarms of faults and fissures, most of which pass through different central volcanoes where volcanic fissure eruptions, silicic rocks and high-temperature geothermal fields are concentrated[2]. Two central volcanoes in this region, Krafla and Askja, have developed calderas. The fault and fissure swarms are arranged *en echelon* subparallel to the north–south direction of the main zone. The northernmost parts of these fissure swarms are intersected in the area of Axafjördur by the Tjörnes Fracture Zone, which has an east–west direction.

The Tjörnes Fracture Zone is a zone of high seismic activity and large earthquakes, most of which seem to be associated with right-lateral strike-slip on WNW striking faults, one of which, the Húsavík fault, has been identified on land, and another, the Grímsey fault, inferred from earthquake location and a fault plane solution. These faults run parallel to each other and are about 40 km apart. The Tjörnes Fracture Zone cannot therefore be viewed as a simple transform fault[2–4]. The present events have been confined to the Krafla caldera, the associated fault swarm to the north and a segment of the Grímsey fault (Fig. 1).

Similar tectonic sequences have occurred before in the Neovolcanic Zone: the last eruption associated with major tectonic movements occurred in 1874–1875 in the Askja caldera and the fault swarm to the north. In 1724–1729 an eruption took place in the Krafla caldera and ground fissures moved in the associated fault swarm.

Attempts made since 1938 to measure ground deformation in this part of the volcanic zone, by measurement of horizontal movement have so far yielded ambiguous results[5,6]. Measurements of vertical movements here since 1966 have shown subsidence of about 10 mm yr^{-1} (ref. 7). Preliminary measurements show that horizontal and vertical ground movement during the present event is at least 2 m.

A geothermal power station (60 MW) is now under construction inside the Krafla caldera, a diatomite factory is located on the fault swarm to the south near Námafjall, and a small village is situated at Reykjahlíd (Fig. 2). A more intensive effort has therefore been made to investigate thoroughly all aspects of the present tectonic events.

Geology of the Krafla–Axarfjördur area

The present activity is taking place mainly within the Krafla caldera and in the Axarfjördur depression. The two areas lie on a fault swarm which is about 100 km long and has a maximum width of 10 km (Fig. 1).

Basaltic fissure volcanism is concentrated mainly within the Krafla caldera and near Námafjall (Fig. 2). In postglacial time about 20 eruptions have occurred in the Krafla caldera and its nearest surroundings, and about 15 in the Námafjall area. Most of the fissure eruptions are basaltic, but andesite and dacite flows have also been erupted in both areas. Voluminous silicic eruptions have not occurred in postglacial time but four subglacial silicic eruptions within and around the Krafla caldera have produced large domes or ridges during the last glacial period. The Krafla caldera contains explosion craters that have ejected small quantities of rhyolitic pumice. The most recent crater formed in 1724 at the beginning of the 1724–29 volcanic episode (Myvatn fires).

Tephrochronological studies[8] indicate that postglacial volcanism in the Námafjall and Krafla areas occurred in two main periods: in early postglacial time, and during the last 3,000 yr. The period of repose may have lasted more than 4,000 yr. The Krafla and Námafjall parts of the fissure swarm have erupted 6–7 times each during the latter period of activity. At least two of the eruptions are common to both areas. Volcanism near Krafla has been concentrated along the central part of the fissure swarm during this period; earlier, it was dispersed over the entire caldera. The largest volume of basaltic lava produced in a single eruption in the area came from the Threngslaborgir–Lúdentsborgir crater row on the Námafjall part of the swarm about 2,000 yr ago. Its total volume has been estimated as 2–3 km^3 and the areal extent is about 220 km^2 (refs 9–11). Few flows of the swarm, however, exceed 0.2 km^3.

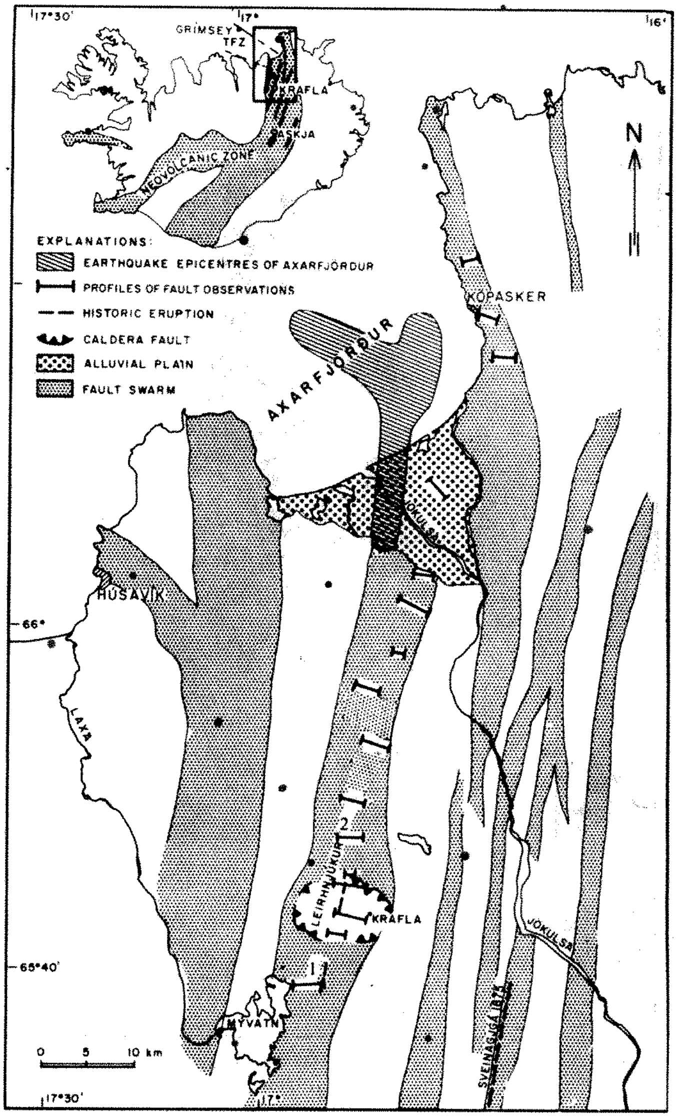

Fig. 1 Fissure swarms in the Neovolcanic Zone of north Iceland. The present rifting is taking place in the swarm through the Krafla caldera and extends from Myvatn to Axarfjördur. Inset map shows the location of the area in relation to the Neovolcanic Zone and the Tjörnes Fracture Zone (TFZ). Dates of activity: 1, 1727; 2, 1725–29, 1746, 1975.

The Krafla caldera measures about 10 km east–west and about 8 km north–south. It formed during the last interglacial period and has since been filled almost to the rim with volcanic material. The caldera's collapse almost certainly followed the eruption of a sheet of dacitic welded tuff which is exposed around the caldera (Fig. 2), and is an airfall tuff, blown mainly towards the north-east from a source near the centre of the caldera. A shield volcano with a diameter of at least 20 km existed before the caldera formation, and its remnants enclose the caldera on the east and west sides, exposing lavas and breccias dipping outward at low angles. Dykes and eruptive fissures trending parallel to the caldera ring fault are conspicuous around the caldera.

A high-temperature geothermal field lies within the caldera. Drilling has revealed temperatures >340 °C at

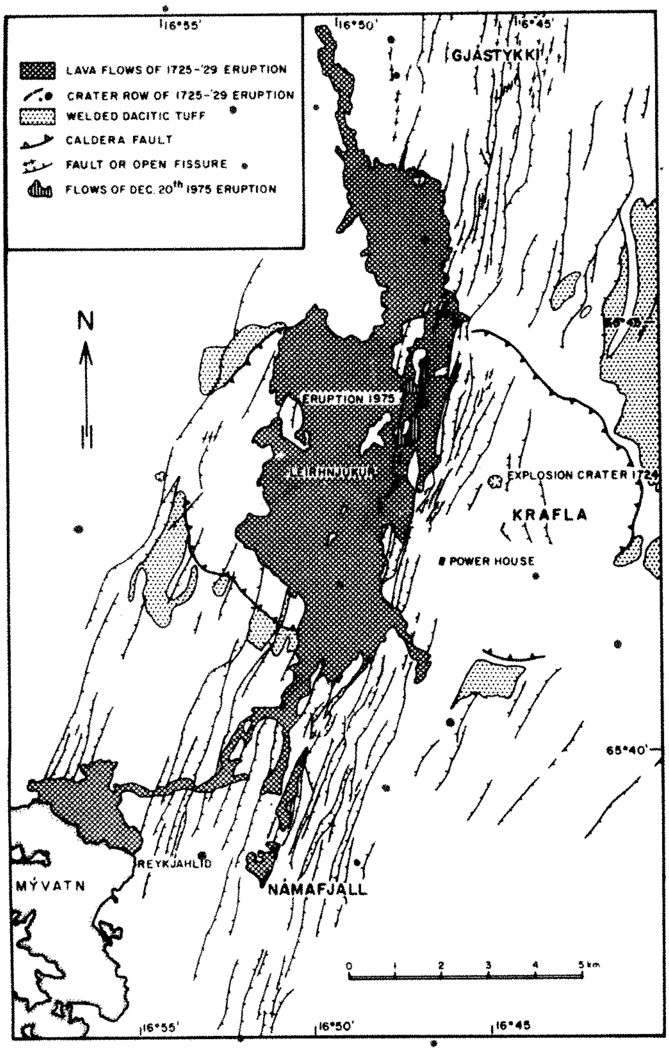

Fig. 2. Outline geological map of the Krafla caldera and the associated fault swarm.

2 km. Basaltic intrusions, with dolerite and granophyre, become increasingly abundant below 1.2 km. Corresponding coarse-grained rocks are conspicuous among xenoliths of the welded tuff and the ejecta of the 1724 explosive eruption. Another high-temperature geothermal field exists at Námafjall, and drilling to a depth of 1.8 km, revealed temperatures of over 290 °C.

Petrological studies in the Námafjall–Krafla area show compositions ranging from olivine tholeiites to rhyolites. Within postglacial time all these rock types have been erupted. The basalts of the fissure swarm through Námafjall and Krafla are quartz normative tholeiites which seem to be of almost identical composition throughout the region.

Axarfjördur is a down-faulted trough 25 km broad that has subsided at least 1,000 m relative to its uplifted western margin at Tjörnes during the Brunhes magnetic epoch[2]. Volcanism is unknown within the Axarfjördur depression but has occurred along its western and eastern margins. The fault swarm which passes through Námafjall and Krafla runs into the eastern part of the Axarfjördur depression.

Historic volcanic activity

Historical accounts mention only one eruption in the Krafla–Námafjall fissure swarm in 1724–1729. Contemporary descriptions of this eruption, the Myvatn fires, are available[12]. On 17 May 1724, a row of explosion craters formed. The eruption was mainly from one main crater, Víti, which ejected both silicic pumice and basaltic scoria, but mostly mud and rock debris from the near-surface rocks[13]. This eruption lasted only a day or two. In 1725, on 11 January, earthquakes occurred and ground fissures opened along the swarm from near Krafla southwards. Steam and mud craters formed in the Krafla and Námafjall geothermal fields, with little or no volcanic activity. Earthquakes were felt throughout 1725. No further significant activity was reported until August 1727, when a fissure eruption started in Leirhnjúkur near the centre of the caldera. A pulse in this eruption occurred in April 1728, when the eruptive fissure extended southwards to Námafjall. Another pulse occurred in December 1728. The lava continued to flow until September, 1729, when the eruption stopped. The lava flows cover about 35 km^2 and the total length of the discontinued volcanic fissure is 11 km (Fig. 2).

A minor volcanic eruption was reported on this fissure in 1746, accompanied by earthquakes and ground movements, but the eruption site or products have not been located.

A similar course of events was observed during the 1874–75 activity of the Askja caldera and the associated fault swarms[12,14]. In February 1874, increased steam emission was observed in the Askja caldera. Earthquakes and fissure movement were first observed during the autumn of 1874, 50 km north of the Askja caldera itself[14]. In January 1875, a small eruption occurred in the caldera, followed in February by a basaltic eruption within the fissure swarm, 50 km north of Askja, which continued intermittently for 8 months, accompanied by intense earthquake activity and considerable fault movement. Within the Askja caldera a maor silicic tephra eruption on 29 March produced approximately 0.5–0.7 km^3 of ash, calculated as solid rock[15], and a caldera of about 2 km^3 volume collapsed within the pre-existing caldera[15].

In 1618 earthquakes and faulting, lasting several months, in the fault swarm west of the Krafla–Axarfjördur swarm were recorded (Fig. 1) (ref. 16).

The initial seismic activity

Conspicuous seismic activity was noted in the Krafla caldera during early 1975. Towards the autumn, when earthquakes approaching magnitude 4 occurred there, it became apparent that this activity was unusually high. The number of seismograph stations was therefore increased, and in October 1975 seven stations were in operation within 60 km of Krafla. Furthermore, a local civil defense committee was set up and an emergency plan was designed in case of a volcanic eruption.

In the morning of 20 December 1975, the seismic activity increased markedly. Shortly after 10 a.m. continuous tremor was seen on the seismograph closest to Krafla, accompanied by an intense earthquake swarm that soon saturated the seismographs. When this increase in activity was noticed an eruption warning was issued to the Civil Defense Authorities shortly before 11 a.m. At 11 : 08 an eruption started near Leirhnjúkur in the centre of the Krafla caldera[17,18].

The vigour of the eruption decreased quickly and at the same time the seismic activity, that started near Leirhnjúkur, propagated northward along the fault swarm. Within 2 h at least 40 km of the fault swarm had been activiated.

The earthquake swarm that followed the eruption continued for several weeks but after the first few hours the activity was mainly confined to two separate areas: inside the Krafla caldera and in the Axarfjördur region (Fig. 1). In both areas the magnitude of the largest earthquqakes continued to increase until mid-January. In the Krafla caldera the largest earthquakes occurred on January 6 and 19 (magnitude 5.0), but in the Axarfjördur region the largest earthquake (magnitude 6.3) occurred on January 13, 1976, and caused considerable damage in the village

Kópasker and vicinity. From 20 December to mid-February, during the most intense earthquake swarm, 36 earthquakes of magnitude 4.5 or larger and six earthquakes of magnitude 5.0 and larger occurred.

The epicentres in the Krafla area during this time were distributed throughout most of the caldera. Most of these earthquakes seem to be related to some process within the caldera itself, rather than the fault swarm that crosses the caldera from south to north. Most of the hypocentres were between 0 and 4 km deep, and a few were as deep as 10 km.

In the Axarfjördur area, however, there was a clear relationship between the distribution of epicentres and known fault systems. Epicentres were distributed along the north–south trending fault swarm from 66°00'N to 66°14'N, where the epicentre belt took a north-westerly trend to follow the Grímsey fault of the Tjörnes Fracture Zone (Fig. 1). The large earthquake on 13 January took place near the junction between these two fault systems.

The eruption and the lava

At about 11 : 25 a.m., 20 December 1975, an eruption was reported in Leirhnjúkur from an overflying aeroplane. At 10 : 25 pilots of the same plane had seen no sign of an eruption in the area. Other observations from aeroplanes crossing the area revealed that the eruption probably started at about 11 : 08 a.m.[17,18]. The first reports and photographs taken at 11 : 25 a.m. show that most of the lava had already been erupted.

The eruptive fissure is about 2 km long but lava was erupted from the northern half only, where three small individual lavas were erupted with a total surface area of 0.36 km^2. The new craters formed on the same fissure as was active during the eighteenth century eruption.

The first visitors to the eruption site at noon saw only relatively minor movements at the lava margin. However, they witnessed fault movements and the opening of a mud crater on a fault which cuts through Leirhnjúkur. In the evening of 20 December, very minor volcanic explosions were observed in the craters; the explosions ceased altogether during the following night. After the lava emission stopped the craters continued to emit water and steam, at first, with considerable force, then rapidly decreasing. Only minor steam vents are still active along the fissure.

The lava is an extremely vesicular pahoehoe type lava, with average thickness of <1 m. No cones were formed and there was no ash production. This lava is homogenous in composition and identical to other basalt lavas that have been erupted from this fissure swarm in postglacial time. It is basalt (quartz normative tholeiite) with occasional glomerophyric plagioclase and rare microenphenocrysts of olivine.

Horizontal and vertical movement

Movement along pre-existing faults was noticed very soon after the earthquake activity began. Some idea of the horizontal and vertical movement could be gained by measuring newly formed cracks. The width of individual cracks was measured and the aggregate movement deduced from measurements on profiles across the fault swarm (Fig. 1). In the Krafla area, rifting and vertical fault movement were both about 70 cm. In the Axarfjördur area, the aggregate widening of the fault swarm was 1.0–1.5 m on 30–40 individual faults[17]. Subsidence within the fault swarm in Axarfjördur is estimated to be at least 1 m.

Field inspection of the fault swarm between Axarfjördur and Krafla at 5-km intervals showed that the major movements were confined to the Axarfjördur district and the Krafla caldera, with minor displacements between these areas. South of the Krafla caldera, fault displacements of 5–10 cm occurred between Námafjall and Lake Myvatn.

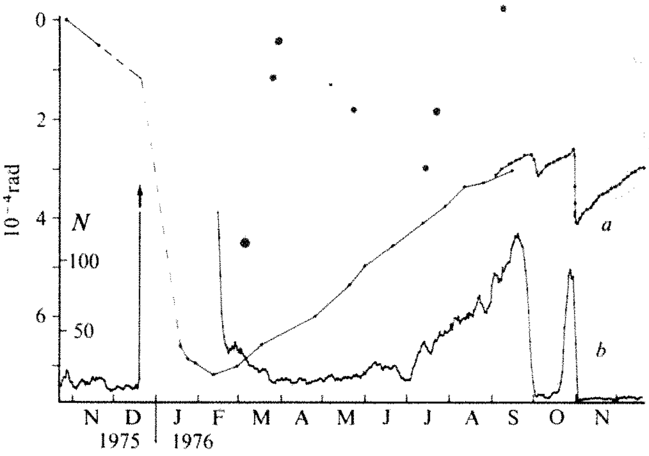

Fig. 3 Correlation between (*a*) the tilt of the Krafla power house and (*b*) the seismic activity within the caldera. The north–south component of tilt is shown, (increasing numbers mean tilt down towards north). The tilt corresponds well with the land elevation. *N* is the 5-d running average of the number of earthquakes per 24 h recorded at the seismic station near Reykjahlíd (Fig. 2) with amplitude above a certain threshold. From 20 December 1975, to 14 February 1976, *N* was >130 and most of that time >1000. The tilt measurements before 20 August were done by optical levelling by engineers of Thoroddsen and Partners, engineering firm.

The fault direction is 20°E. Most of the faults indicate a simple opening movement, but occasional faults have vertical displacements of up to 60 cm. Movements connected with the large Kópasker earthquake of 13 January affected faults east of Axarfjördur, although the epicentre of the earthquake was off the coast.

Levelling carried out in the southern part of the Krafla area in 1974, and in early March 1976, indicated that considerable subsidence had occurred. The maximum measured subsidence of 2.1 m relative to the north end of Lake Myvatn was found near the eruption site. Further, it was noted that a 70-m long building at the power station inside the southern part of the caldera (Figs. 2 and 3) had tilted about 700 μrad to the north relative to measurements made 2 months before the eruption.

Later events in the Krafla area

After the culmination in January 1976, seismic activity gradually decreased in both the Axarfjördur and the Krafla areas. In March 1976, the events in the Krafla area took an unexpected course and since then there has been a significant interplay between land elevation changes, fault movements, earthquakes and bursts of volcanic tremors within the Krafla caldera and the associated fault swarm.

Soon after the large earthquake swarm was over, the area started rising again (Fig. 3). The rate of uplift was relatively constant, 6–7 mm d^{-1}, at its maximum near the centre of the caldera, decreasing gradually outwards to <1 mm d^{-1} 10 km south of the centre. In May, a slow increase in the frequency of earthquakes was noticeable. Measurements showed that the land had regained 50% of the original subsidence by the end of September, and the earthquake frequency had increased by a factor of 10 as compared with the minimum in April. Then a sudden drop in the seismic activity was noticed and at the same time subsidence occurred at a relatively high rate, lasting 6 days and amounting to about 15 cm near Leirhnjúkur. During the period of highest subsidence rate, bursts of continuous tremor were seen on the seismographs and small earthquakes were recorded, originating in the fault swarm about 15 km north of the caldera, on the Gjástykki faults. On 4 October, the subsidence stopped, and the area started rising again at a similar rate as before.

The seismic activity remained very low until the land elevation approached the same level as before the last subsidence. Then the earthquake frequency increased sharply to almost the same level as before. The elevation had slightly passed the level of 30 October, when further subsidence started, lasting about 48 h, and the area around Leirhnjúkur subsided about 50 cm. The subsidence rate was much higher than in the previous event and was accompanied by continuous tremor on the seismographs. Simultaneously, fissures in the middle of the caldera showed significant contraction. About 5 h after the beginning of the subsidence a large earthquake swarm (not shown in Fig. 3) started again in Gjástykki north of the caldera. People travelling in Gjástykki felt earthquakes and continuous tremor and saw a cloud of steam emanating from a fissure. Later inspection revealed fresh fault movements and a new thermal area in Gjástykki. The earthquake swarm lasted 2 d, and as soon as the earthquakes stopped in the Gjástykki faults the land started rising in the Krafla caldera, this time at a similar rate to before. The earthquake frequency within the caldera is, in late December 1976, at a minimum, but is expected to increase sharply when the elevation approaches the level of 30 October.

Boreholes have recently been drilled down to 2 km in the Krafla area. One of these had been blowing for 4 months before the eruption. In April 1976, the CO_2 content of the aquifer water had increased from 200 to 20,000 p.p.m. The CO_2 content of the borehole has since lowered somewhat but is still 10–20 times higher than before the eruption. At the same time, the *p*H dropped from 8.6 to 1.9 in the discharge from another uncontrolled borehole, and has since returned to its previous value.

The composition of gas emitted from fumaroles has changed significantly towards increase in CO_2, relative to H_2S and H_2. The appearance of new fumaroles and increased activity of others indicates a significant increase in the thermal output of the area during the last few months.

Boreholes at Námafjall extend down to 1.8 km, and a considerable amount of data are available on these holes. Measurements since the events of 20 December 1975, have so far revealed no change in composition compared with earlier measurements.

Discussion

The main significance of the activity taking place now in northeastern Iceland is the light it throws on the mechanism of rifting and the associated magmatism. It demonstrates that the rifting long predicted for Iceland as a part of the oceanic rift system is episodic rather than continuous. The historical record shows that the interval between these episodes is of the order of 100–150 yr. Each rifting episode has a duration of several months to a few years, and extends over 50–100-km distance along the rift zone. The present episode started near the south end of the zone that was activated but propagated rapidly along the fault swarm. Seismic activity and faulting have since been primarily concentrated near the ends of the active rift; the central volcano Krafla in the south and the intersection of the North Iceland Rift Zone and the Tjörnes Fracture Zone in the north. Seismic energy release has been greater near the northern end of the zone than near its southern end, but the northern end now seems to be coming to a rest while the southern end is still active 12 months after the rifting started.

This rifting, like that in the eighteenth and nineteenth centuries, is closely connected with magmatism. The causal relationship between rifting and magmatism is, however, unclear.

The eruption of 20 December 1975, is only a minor manifestation of the total magma movement. The volume of the lava erupted (3×10^5 m^3) is much smaller than the volume of the initial subsidence bowl, which can be estimated at 10^8 m^3. The subsidence is interpreted as a result of the widening of the rift system and a movement of magma from the Krafla area along the fault swarm to the north, presumably along a horizontally fed dyke opened by the rifting process.

Later, in March 1976, the Krafla area started inflating, but the inflation has twice been interrupted by short subsidence events. At the end of September and the end of October (Fig. 3). These subsidence events are of a similar nature and it is probable that the difference between these and the large event that started on 20 December 1975, is only in degree.

The inflation is interpreted as being caused by magma transported from below accumulating at a shallow depth within the Krafla caldera. Simple model calculations indicate that the inflow of magma into the roots of the caldera since March is approximately 5 m^3 s^{-1}.

The deflation is caused by release of pressure in the magma chamber by widening of the rifts and flow of magma into the fault swarm north of the caldera. This flow is accompanied by volcanic tremors, earthquakes, faulting and thermal activity in the fault swarm. In this model the earthquakes in the caldera are caused by inelastic deformation of the crust above the magma chamber.

Further support of this model comes from work in progress on the distribution of earthquake hypocentres within the caldera and the attenuation of S waves, which strongly suggests the presence of a magma chamber below the depth of 3 km. The low *p*H value measured in the borehole fluid last April and the increase in CO_2 are interpreted as being a result of the influx of magmatic gases into the hydrothermal system.

Judged from previous historical events, years may pass before the area comes to rest. Therefore, and because of the economic importance, great emphasis is put on continued monitoring of the area, including telemetering of signals from three seismometers to an observatory where they are watched around the clock. The tilting of the Krafla power house is recorded twice a day and the ground movement is measured by optical levelling and gravimetry every 2–4 weeks. The movement of faults is monitored. The composition of borehole discharge is monitored regularly. The gases emitted from fumaroles are analysed every few weeks. Ground temperatures are measured and a close watch is kept on the thermal fields. Plans have been tested so the area can be evacuated at a very short notice in case of abnormal seismic activity.

From the data accumulated so far a better understanding of the rifting mechanism and the associated magmatism is rapidly emerging. It is obvious already that the data will reveal many of the detailed features associated with the formation of the Icelandic crust.

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Note added in proof: On 20 January 1977, the floor of the Krafla caldera subsided again about 30 cm in 20 h. This was accompanied by seismic activity, fault movements and development of a new steam field within the fault swarm, Gjástykki. The centre of the caldera started to inflate immediately after this subsidence and another subsidence period is expected in early March 1977.

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Saturated platelets are new intermediates in hydrocarbon pyrolysis and carbon formation

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Formation of solid carbon during pyrolysis of gaseous hydrocarbons may involve 'saturated platelet' intermediates.

AN important problem in flames and in the industrial pyrolysis of hydrocarbons is the understanding of how solid carbon is formed, either in the gas phase (soot) or on surrounding surfaces. Various mechanisms have been discussed[1,2], but none takes full account of soot ball internal structure, electrical phenomena and the startling temperature range (1300–2300 K) of soot formation. This article proposes a single route to soot for all conditions, passing through sequential chemical and physical steps.

Small intermediate molecules

To explain soot formation from varied starting hydrocarbons, possible common intermediate molecules proposed are carbon vapour (C, C_2, C_3, and so on), benzene (and other aromatics), and acetylene[3,4]. Gaydon and Wolfhard[1] have convincingly criticised the carbon vapour route at lower temperatures. Certainly the band radiation of C_2 is not evident in pyrolysing hydrocarbons at lower soot-forming temperatures[5]. It has been suggested that benzene and other aromatics condense chemically to form the polyaromatic molecules found in flames[6], and hence soot. During pyrolysis of benzene, however, benzene absorption disappears well before soot-like luminosity is seen, without the appearance of polyaromatic bands[5]. Concentrations of polyaromatics in a premixed flame continue to rise slowly through the sooting region, indicating that polyaromatics are byproducts rather than intermediates of the sooting process[7].

Porter[3,4] states that acetylene is "the last stable hydrocarbon to be observed before carbon formation". It is formed in appreciable quantities in most pyrolyses above 1300 K, and is still relatively stable at the highest soot-forming temperatures (2300 K). Temperature measurements of the pyrolysis of methane show that an overall endothermic process is followed by an overall exothermic process[8]. This is consistent with strongly endothermic reactions to acetylene, followed by exothermic steps to soot. Porter has outlined processes which would begin with acetylene and end with soot. Obviously some polymerisation will occur ($C_2 \rightarrow C_{10^6}$) and some dehydrogenation (H/C from 1 to 0.1 atom ratio), but the sequence of these steps will be governed by the chemical kinetics. The high transition-state entropy decrease found with typical polymerisations, and the higher activation energies found with decompositions suggest that most decomposition reactions will be faster than polymerisations for temperatures higher than 1300 K (ref. 3). Porter concluded that little polymerisation could occur at sooting temperatures without some dehydrogenation, and that they were simultaneous processes and intimately connected.

Saturated platelets

If acetylene is the common small intermediate in soot formation, it is probable that there are larger intermediates formed before soot, for a study of soot formation in heated acetylene showed that the soot balls continued to grow in size even after all the acetylene had been removed[9]. Optical absorption evidence in an oxygen–acetylene flame showed that all the soot substance was present in some larger (absorbing) intermediate before much soot was detected[10], and cooled-probe tar collection in benzene pyrolyses[11] gave similar indications.

A continuum absorption has been seen in pyrolyses and diffusion flames of hydrocarbons[5]. This absorption was probably due to larger soot precursors or intermediates because it was seen in all pyrolyses and flames before sooting occurred, and it was not seen in the soot-free pyrolysis of methyl alcohol. These precursors of soot are probably generally formed from acetylene, for the continuum was found at lower temperatures in the pyrolysis of acetylene than with other hydrocarbons (including benzene). In a mass spectrometry study of transient species in low-pressure acetylene–oxygen flames[7], two types of species peaked in concentration before soot was found—polyacetylenes ($C_{2n}H_2$), and a group of much larger molecules in the mass range 300–500. These latter were found in very low concentrations (10^{-7} mole fraction) compared with the polyacetylenes, suggesting a route through polyacetylenes to soot, although the larger molecules could also contribute. Polymerisation of long rod-like polyacetylenes at higher temperatures seems unlikely, however, as configurational entropy and activation energy differences probably again favour decomposition reactions. Mass spectral data suggest that the larger molecules lost hydrogen continuously before or during sampling. By collecting 'young soot' on cooled tubes in the flame, the hydrogen–carbon ratio of the substance was found to be as high as 1.

These observations can be explained if one postulates that structures of the type shown in Fig. 1 are formed directly from acetylene. They retain the H–C ratio of acetylene at lower temperatures, and have a puckered layer of carbon atoms bonded by single bonds, except at the edges. Hence 'saturated platelets' is an appropriate term for them. They can retain radical sites, and acetylene can add on by a radical mechanism. Hydrogen atoms are held above and below the carbon layer on alternate carbon atoms, and can be lost as H_2 from the interior of the platelet by a radical mechanism. Hence graphite-like layers are formed from the inside, moving to the outside, and simultaneous addition of acetylene and dehydrogenation is possible in the same molecule at different sites in excellent agreement with Porter's prediction. If the dehydrogenation is more rapid than addition, only a perimeter of hydrogen atoms remains and one has the polycyclic aromatics observed in flames. Acetylene pyrolysis in conditions where no breakdown of the carbon skeleton of platelets is expected[12] (small hot wire immersed in cold acetylene) yielded an oily deposit on the walls, consisting of "a series of heavy polynuclear aromatics containing even numbers of carbon atoms". This is good evidence for the direct build-up of acetylene into these larger species.

Evidence supporting the existence of saturated platelets is the observation[13] of arrays of 0.154-nm (singly-bonded) C–C bonds by electron diffraction of 'young soot', in addition to the

0.141-nm graphite-like C–C spacings. Also, 'young' soot has 100 times the electron spin resonance strength of 'old' soot sampled further downstream in the flame[7], indicating many radical sites on the young soot. This is consistent with the presence of saturated bond areas on the adhering material which neutralise free radicals in the process of dehydrogenation to the more stable aromatic form.

The thermodynamics of platelets, calculated from group properties, can be summarised thus (see also note added in proof)

$$\begin{array}{llll}
\mathrm{C_2H_2} \xrightarrow{1} 2\,(\mathrm{CH})\ (\text{saturated platelet}) & & \xrightarrow{2} 2\mathrm{C}\ (\text{graphite}) + \mathrm{H_2} & \\
\Delta H_1^\circ = -240\ \mathrm{kJ\ mol^{-1}} & & \Delta H_2^\circ = 15\ \mathrm{kJ\ mol^{-1}} & \\
\Delta S_1^\circ = -360\ \mathrm{J\ mol^{-1}\ K^{-1}} & & \Delta S_2^\circ = 220\ \mathrm{J\ mol^{-1}\ K^{-1}} &
\end{array}$$

all at 300 K. ΔG_1° is negative for temperatures < 650 K, whereas ΔG_2° is negative for all temperatures > 70 K. Saturated platelets can then be expected to be short-lived at flame temperatures before dehydrogenation occurs. The kinetics of acetylene disappearance and the appearance of ethylene, benzene and so on, at the lower temperatures has been interpreted in detail in terms of radical platelets. This analysis will be published elsewhere, but it is sufficient to note here that the 'polymer' of composition $C_{n2}H_{n2}$ found by Silcocks[14] in particular corresponds well with the existence of saturated platelets.

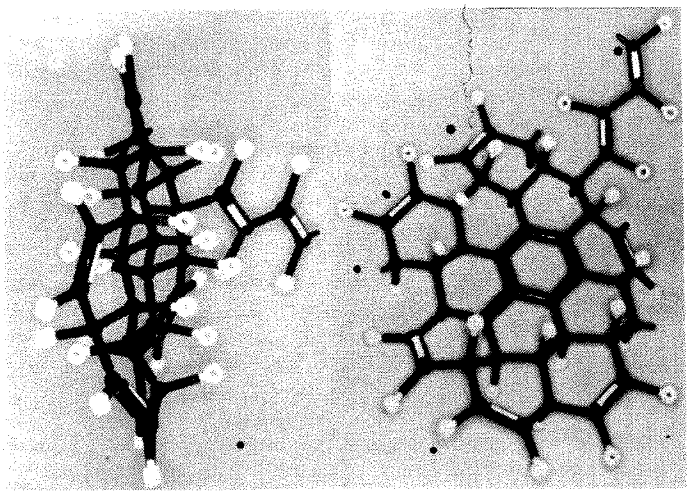


Fig. 1 *a*, Model of a $C_{27}H_{27}$ saturated platelet, with attached C_4H_4 radical chain, showing the puckered carbon skeleton and distorted general shape. The $C_{27}H_{27}$ heat of cumbustion $Q = 330$ kJ mol^{-1} C atoms, equal to that of a bituminous coal[14]. *b*, Model of a $C_{38}H_{38}$ platelet with attached C_4H_4 radical chain, showing a central aromatic region formed by dehydrogenation. The $C_{38}H_{38}$ $Q = 365$ kJ mol^{-1} C atoms, higher than that of a bituminous coal[14,15] but accounting for several C–O bonds restores Q to that of coal.

Control of platelet formation and growth

Formation of saturated platelets probably begins with the formation of benzene (or a derivative) and then naphthalene (or a derivative). Experiments with nitric oxide[14] indicate a radical mechanism for acetylene disappearance, so a phenyl radical and then larger radicals are likely intermediates on the route to a saturated platelet radical (Fig. 2). The disappearance of acetylene can, in fact, be explained only by the involvement of a large molecule. The kinetics of disappearance are second order over the extraordinary temperature range 650–2500 K (ref. 15). Bimolecular mechanisms which are currently favoured[15] utilise small radicals such as C_2H, especially at high temperatures. Reactions with five to seven atoms in the transition state, however, become energy-transfer-limited at temperatures in the vicinity of 1000 K (ref. 16). Hence a small radical–acetylene reaction which was second order at lower temperatures should become third order at temperatures not much higher than 1000 K. If the radical is attached to a large enough body (platelet), the decomposition should remain second order to very high temperatures, as observed.

Soot formation may be limited at the higher temperatures by the initial steps of producing phenyl (C_6H_5) radicals or similar aromatic radicals, as expected from entering an energy-transfer-limited regime. This would explain why, in premixed flames, naphthalene and benzene derivatives produce soot at lower fuel–air ratios than acetylene[1]. This 'sooting tendency' in competition with oxidation has been used to argue for the aromatic polymerisation route, and against the acetylene mechanism, but the survival of some aromatic radicals derived from benzene or naphthalene fed to the flame may bypass the otherwise limiting step of making benzene or naphthalene and allow a faster build-up of acetylene on these radicals to form saturated platelets.

In shock tube studies[17] of benzene, toluene, ethylbenzene and indene in the temperature range 1600–2300 K, soot particles were identified by the scattering of light of 488 nm wavelength, but there was also absorption of this light in the pyrolysing mixture before scattering occurred. There was absorption at 632.8 nm which increased later than that at 488 nm, entirely consistent with the absorption found by Parker and Wolfhard[5]—a continuum spreading from the ultraviolet into the visible spectrum. Measurements of the ratio of absorption at these two wavelengths[17] show that the ratio reached a steady value (when dehydrogenation had ceased) close to the time soot particles appeared. Again, these observations are consistent with the formation of saturated platelets which are dehydrogenated to the point where they are agglomerated to soot particles. The continuum spreading into the visible is likely to arise from increasingly conjugated double bonds, either in chains attached to the platelets or integral with its structure, on an edge.

The finding that this absorption reached a plateau which was lower (lower soot yields) with higher temperature in contrast with the higher rate of absorption increase with higher temperature, was interpreted[17] as indicating competition between a rapid direct soot formation from aromatics, and a slower indirect route to soot by fragmentation reactions (for example, through acetylene). An alternative explanation is that the absorption measured platelet concentration rather than soot during its inital increase, and that initiation of platelets, which occurred almost solely at the beginning of the shock period when hydrocarbon concentrations were high, has a negative temperature coefficient because of a rapid route through an aromatic (unstable) vying with a slower acetylene (stable) initiation. Thus, as the temperature was raised, fewer platelet initiations occurred, resulting in a lower conversion to absorbing platelets and soot. Growth of each platelet was more rapid with rising temperature, however, showing a more rapid rise of absorption.

Agglomeration of platelets

A mechanism proposed for agglomeration of larger intermediates is physical nucleation and condensation into liquid droplets. This was supported by Lahaye, Prado and Donnet[18]. If liquid condensation occurs at all, however, it must be limited to the very lowest of sooting temperatures. The large molecules found by Homann and Wagner[7] correspond to C_{25}—C_{40} polycyclics. The condensation points of these molecules can be estimated—the polycyclic perhydrochrysene ($C_{18}H_{30}$) has a boiling point (1 atm) of 630 K (ref. 19) and the condensation point of the C_{40} molecules is likely to be no greater than 1100 K (by extrapolation of Fig. 35 from ref. 20). Johnson and Anderson[9] collected 'polymer drops' from the pyrolysis of acetylene, but these only appeared when they sampled in regions with the temperature below about 570 K. They concluded that any drops observed after pyrolysis had been condensed on to small carbon particles and then only when the pyrolysis gas was cooled below 570 K. It is thus unlikely that a liquid droplet route can operate in the formation of soot balls.

A number of observations support an electrical mechanism of soot ball nucleation. An increase in soot particles deposited on electrodes from a diffusion flame was found after seeding with cesium chloride[21]. When soot formation was promoted and positive hydrocarbon ion concentrations were elevated by adding carbon tetrachloride in a diffusion flame[22], there was a local depletion of ions where soot was forming. The concentrations of large positive hydrocarbon ions in premixed flames have been shown to peak just upstream of soot formation[23], and many of the younger soot particles were positively charged[24].

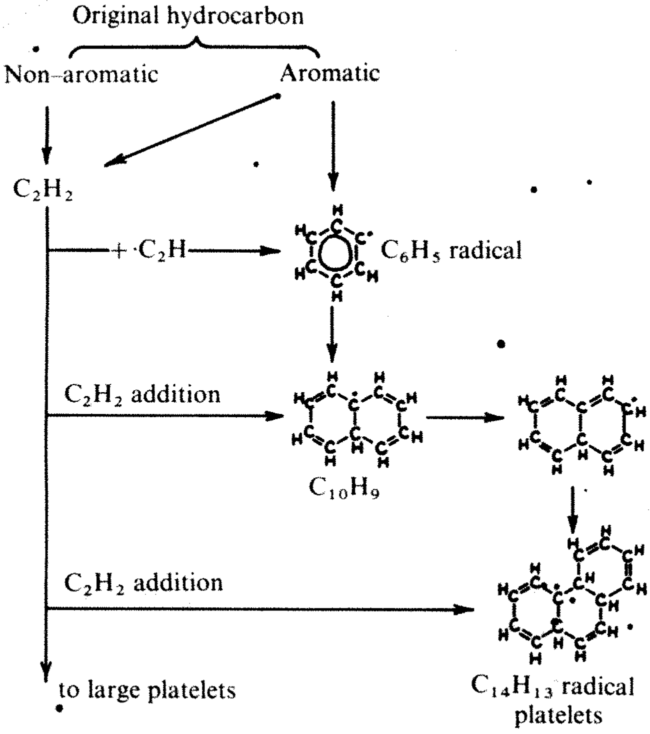


Fig. 2 Initial steps in the formation of a saturated platelet by radical addition of acetylene.

The necessity of electrical charges playing some part in soot formation has been challenged[1,25] with the observation that in the absence of a flame and its non-equilibrium ions produced by chemi-ionisation, ionisation should be negligible, and a pyrolysing hydrocarbon is not expected to soot by an electrical mechanism. There is electric probe evidence of charge carriers being generated in pyrolysing hydrocarbons in the absence of a flame in heated propane and methane[26]. Above 1330 K the probe current increased rapidly and carbon deposited only on the anode; during ethylene pyrolysis[27] at the lower temperature of 1100 K, with much higher electric fields, carbon deposited only on the cathode. Current transients from a probe immersed in shock heated ethylene indicated an ion recombination after the initial ionisation. These studies indicate that a flame is not necessary for the appearance of charge carriers related to soot formation, and suggest that the ions formed by the oxidation process itself are not necessary for some, at least, of the soot formation in flames.

At higher temperatures it seems that negative charge carriers were carrying more carbon than positive charge carriers[26], suggesting the existence of large negative hydrocarbon ions. Ion-pair processes $AB \rightarrow A^+ + B^-$, or ionisation $A \rightarrow A^+ + e$ followed by a rapid capture of the electron may well occur in hydrocarbons at these relatively low temperatures. Possible steps involve $C_3H_3^+$ ($I = 5.8$ eV, refs 28, 29) and C_2H^- (E.A. = 2.2 eV, see ref. 30), both of which have been found in flames[31,32]. Dehydrogenating platelets, however, have the possibility of creating aromatic edge sites with σ-electron affinities similar to those of C_6H_5, $C_{10}H_7$, $C_{14}H_9$ radicals (2.2 eV or 1.2–1.6 eV (refs 33 and 34)). Thus negative platelet ions are good candidates for the large ions mentioned above.

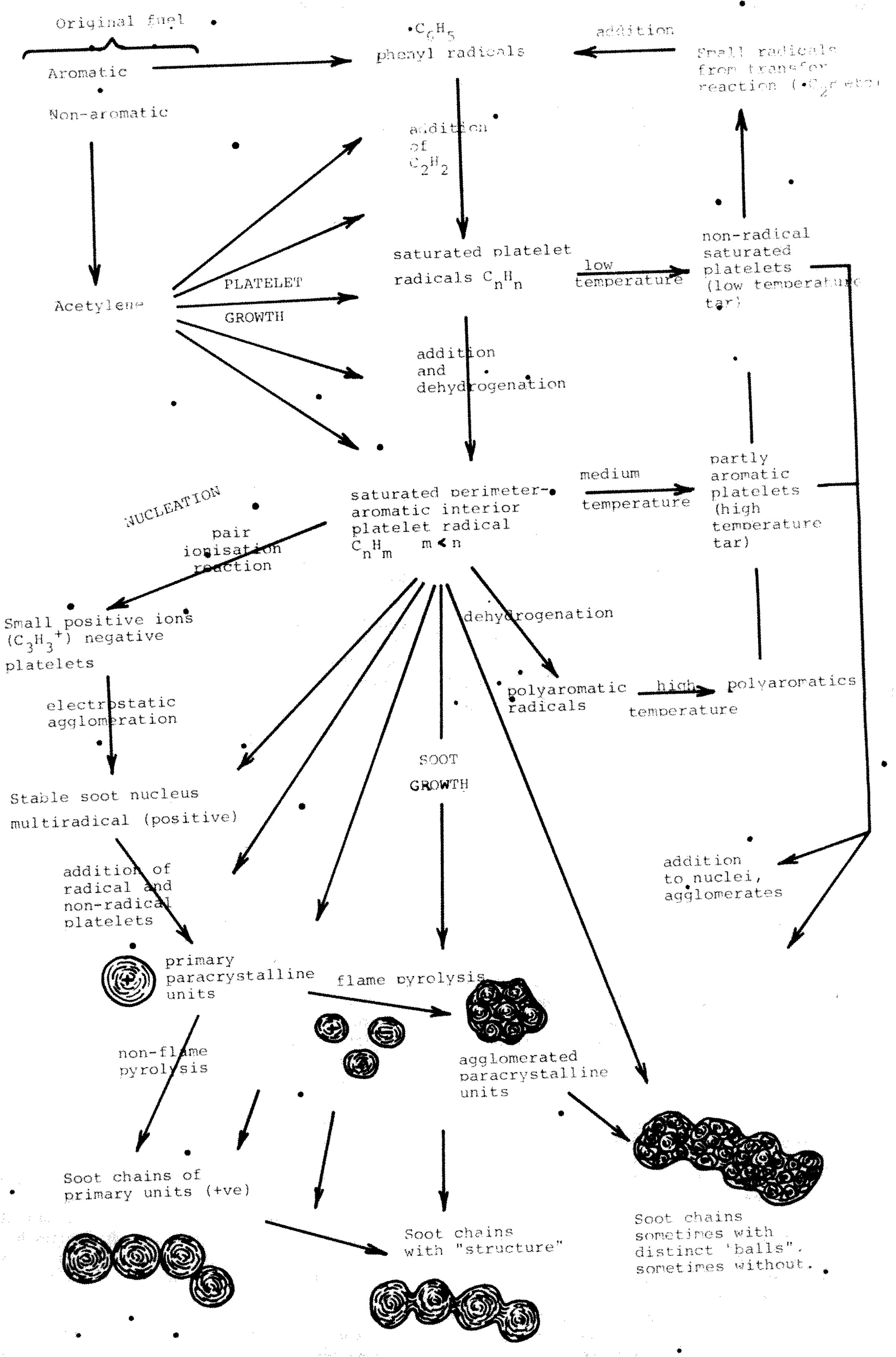
Evidence for generation of large negative hydrocarbon ions in pyrolysing hydrocarbons is indirect because studies have been done only in flames, for which an alternative interpretation is available—that electrons diffuse to the pyrolysis zone from the combustion zone, and attach to form negative ions. Large (up to the mass spectrometer limit, 130 mass number) negative ions have been found[35] on the fuel side of a diffusion flame. The validity of the electron diffusion interpretation is being checked by a similar study on pyrolysing hydrocarbons.

Thus, let us assume that a weak plasma exists in a pyrolysing hydrocarbon, consisting of small positive ions, large negative platelet ions and neutrals. Some of the neutrals will be platelets, and a certain fraction of all platelets and smaller molecules will be radicals. It is clear that this combination can lead to electrical-radical nucleation.

(1) Some radicals will attach ions, and on average will attach positive ions more frequently than negative ions (the positive ions are smaller and more frequently bombard other species because of their high velocities).
(2) The predominantly positive radical-ions attract and hold a negative platelet by an electrostatic force, resulting in a directional approach and instantaneous bonding.
(3) The resulting neutral radical will on average acquire another positive charge by process (1) (bombardment charging).
(4) A further electrostatic neutralisation as in (2) will occur.
This cycle will be repeated until sufficient radical sites will have been acquired so that neutral platelets begin to be attached (chemically) in appreciable numbers. The nucleation has then ended, and normal growth of the soot ball has begun, with the charge still predominantly positive, but having little influence on the growth. A factor which could also be important in maintaining the positive charge of nuclei relative to smaller gaseous positive ions is the polarisation energy of the packed platelets (that of aromatic crystals is about 1.5 eV (ref, 36)), which will reduce the energy of any ion-pair process originating from the nuclei.

Nuclei growth rate in the above ion-recombination process was calculated for the conditions of Prado's pyrolysis of benzene[11] at 1380 K; this gives a platelet collection rate of only 10^{-4} of the average rate required to build up the final 35-nm particles found by Prado. It seems that the growth accelerates after the nucleus is formed, and that the nucleus is very small, for the final particles contain material equivalent to 10^4 platelets. Notwithstanding the large uncertainties in ion concentrations, these conclusions should still hold. It is difficult to determine whether the growth is wholly irreversible. We have evidence from rapid quenching experiments (unpublished) that a gas phase equilibrium is obtained during soot formation which ignores the presence of solid carbon. This would support an irreversible growth, much slower than the prevailing gas kinetics. On the other hand, there must be competition between nuclei for platelets, and in the very early stages where the number of successful nuclei is determined and the process is unlikely to be irreversible, the electrical sphere of influence of each ion may be important. Thus the Debye shielding radius, or the much smaller ion-recombination radius, may determine the final number of soot balls. For Prado's condition[11], Debye radius calculations gave 2×10^{16} m^{-3}, compared with an experimental 10^{15} m^{-3}.

The physical form of soot is consistent with the platelet agglomeration ideas presented above. High resolution phase-contrast electronmicroscopy[37] has shown that the basal layers in soot particles are aligned parallel to the surface (onion-like), which is easily explained by the wrapping around of platelets deposited from the gas phase. Some layers show interlayer spacing well in excess of graphitic spacing, which is what would be expected from those remaining saturated platelet regions. 'Thermal' carbon blacks—made by the pyrolysis of hydrocarbo in the absence of air—show uniform spherical particles with


Original fuel
Aromatic
Non-aromatic
•C$_6$H$_5$ phenyl radicals
addition
Small radicals from transfer reaction (•C$_2$H etc)
addition of C$_2$H$_2$
Acetylene
PLATELET GROWTH
saturated platelet radicals C$_n$H$_n$
low temperature
non-radical saturated platelets (low temperature tar)
addition and dehydrogenation
saturated perimeter-aromatic interior platelet radical C$_n$H$_m$ m < n
medium temperature
partly aromatic platelets (high temperature tar)
NUCLEATION
pair ionisation reaction
Small positive ions (C$_3$H$_3$$^+$) negative platelets
dehydrogenation
polyaromatic radicals
high temperature
polyaromatics
electrostatic agglomeration
Stable soot nucleus multiradical (positive)
SOOT GROWTH
addition of radical and non-radical platelets
addition to nuclei, agglomerates
primary paracrystalline units
flame pyrolysis
agglomerated paracrystalline units
non-flame pyrolysis
Soot chains of primary units (+ve)
Soot chains with "structure"
Soot chains sometimes with distinct 'balls", sometimes without.

ig. 3 Steps proposed here in the production of soot from a hydrocarbon fuel. At high temperatures (~ 2000 K) the initial steps are rate ontrolling, whereas at lower temperatures (~ 1300 K) the electrical stages are controlling. Production of methane and other light hydrocarbons on radical platelets is not shown.

single centre of growth. In contrast, furnace and channel blacks—made in the presence of air—show many more growth centres per unit mass, and these are firmly embedded together by more onion-like layers[37] around the outside. Wersborg *et al.*[23,24] observed this latter type in flames, and from the peak in particle numbers with distance it seemed that each growth centre had an independent existence in the gas phase before coming together. The different physical forms may be related to electrically aided or hindered agglomeration in that non-flame pyrolysis probably results in many soot nuclei maintaining a positive charge, hindering agglomeration, whereas in flames the soot nuclei may be positive, neutral or negative[24], encouraging earlier agglomeration.

The single-centred or multicentred units are finally found in many soots more or less tightly joined together in long chains. Howard[38] described well this final step of agglomeration, on the basis of the directional agglomeration of repelling like charges. All the steps proposed for soot formation are presented in Fig. 3.

Deposition of carbon on walls at high temperatures

The deposition of platelets on walls is likely to be important wherever the hydrocarbon gas phase has been heated sufficiently to generate acetylene. Examples are the conditioning and coking of thermal cracker walls[39], the deposition of laminated pyrolytic graphite on hot surfaces, and the extensive rearrangements of structure occurring within hydrogen bearing carbon materials on heating. In the heating of cokes, for example, acetylene formed from residual hydrogen can redeposit from the pore spaces as platelets, which will then constitute a graphitisable portion of the solid.

Many hydrocarbon pyrolysis experiments have revealed the importance of aged walls (platelet-covered walls) for reproducibility of rates. The generation of wall radical sites, radical transfer to gaseous species, and eventual termination perhaps back on the wall as radical platelets, can explain such behaviour, and the occurrence of induction periods. The importance of wall-derived radicals is relatively easily recognised, but electrical effects must also be considered. Abrahamson and Kennedy[26] found some electrical behaviour with their metal probe which was interpreted as being surface ionisation, at lower temperatures than that at which appreciable gas phase ionisation occurred. This wall behaviour is consistent with the crystal polarisation effect, whereby the energy of an ion-pair reaction involving a platelet is smaller if that platelet has its electron levels banded with other nearby platelets, as on a wall deposit. The electrical control of wall carbon deposition found by Abrahamson and Kennedy at slightly higher temperatures probably relates only to the initial stages of deposition, but it could well be useful in the commercial control of wall coking.

Deposition of carbon on walls at low temperatures

If wall surfaces can supply free radical sites, and chemisorb products of surface rearrangements, it is possible that saturated platelets may form on the wall surface itself, from small molecules other than acetylene. This route should be observable, if at all, at lower temperatures where acetylene and gas phase platelet formation are unlikely.

Lobo and Trimm[40] measured deposition rates in the range 650–770 K and found zero order kinetics with a common activation energy for deposition from acetylene, ethylene, propylene and butene. Again, a reproducible rate was obtained only after several[41] carbon layers had been deposited. Plummer[42] notes that the surface residue formed on nickel and tungsten has the same photoelectron spectra whether deposited from acetylene or ethylene. The residue formed from ethylene above 230 K. On heating to 500 K, hydrogen desorbed from the surface until a $(C_2H_2)_n$ complex remained, which was stable up to 1100 K. The formation of acetylene and hydrogen chemisorbed on to the surface—one interpretation of these experiments—is not likely to be energetically as attractive as the formation of saturated platelets $(C_2H_2)_n$ together with locally chemisorbed hydrogen. The electronmicroscope observation[43] that pregraphitic deposits formed on platinum catalyst from various hydrocarbons at 633 K covered only distinct tiny patches of the surface supports platelet formation.

These temperatures are similar to those expected for coal formation, and humus formation in soils. I will argue in a later publication that the saturated platelet is the archetypal molecule for both coals and humic substances, and that light hydrocarbons from rotting vegetable matter rearrange relatively rapidly on finely divided soil constituents to form humus, but slowly over extended periods on other platelets to form coal seams.

I thank the Nuffield Foundation for a Commonwealth Travelling Fellowship, and Professor J. F. Davidson and his staff in the Chemical Engineering Department, Cambridge, for their hospitality.

Note added in proof: A recent crystalline orbital calculation[46] of hydrogen atoms chemisorbed on to a single graphite layer (one side only) indicates that saturated platelets should be stable to temperatures of 300 or 400 K, rather than 70 K.

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Sequence and structure of D-glyceraldehyde 3-phosphate dehydrogenase from *Bacillus stearothermophilus*

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The glyceraldehyde 3-phosphate dehydogenase holoenzyme of Bacillus stearothermophilus *possesses precise 222 symmetry: in this respect it differs from the reported structure of the lobster muscle enzyme. Pairs of active sites are linked through a flexible polypeptide loop which probably mediates the structural changes giving rise to cooperative effects. Three additional salt bridges made by each subunit to others would make a major contribution to thermostability of the tetramer.*

MANY current ideas about the cooperative nature of protein transitions have derived from studies of D-glyceraldehyde 3-phosphate dehydrogenase (GPDH). The structure and mechanism of this enzyme have been investigated for several species (for a review see ref. 1). All known GPDH molecules are tetrameric with a molecular weight of 145,000 and bind four moles of the coenzyme, NAD. Although the four subunits are chemically identical[2] the affinity of NAD for the muscle enzyme decreases with each additional NAD bound. The first two moles of NAD are bound tightly and the second two loosely[3-5]. Yeast enzyme, however, displays a complicated mixture of positive and negative cooperativity[6,7].

The model which best describes the binding is controversial. The allosteric model[8] of cooperativity and the induced fit model[9-10] differ with regard to the preservation of symmetry at various states of ligation. Both models assume complete symmetry for the non-ligated and fully ligated states, but the induced fit model allows asymmetry for intermediate states. A model for GPDH has been proposed[11] in which the molecule is never fully symmetric, but consists of an asymmetric pair of symmetric dimers, and this view has received support from crystallographic studies of the lobster enzyme[12].

The enzyme from mesophiles becomes unstable once NAD is removed. Because of its greater stability, GPDH from the thermophile *Bacillus stearothermophilus* can be crystallised with a range of NAD stoichiometries from zero to four moles per tetramer[13]. The structure and properties of the bacterial enzyme are very similar to those of the muscle enzyme[14-16].

We report here the complete sequence of 333 amino acid residues in the subunit polypeptide together with the interpretation of an electron density map at 2.7-Å resolution of the holoenzyme with four moles of bound NAD. For the first time it is possible to compare the structure and sequence of a multisubunit protein from both a mesophile and a thermophile. Our structure differs in some important respects from the structure of the lobster muscle enzyme[12,17,18], especially with regard to molecular symmetry.

Experimental methods

B. stearothermophilus GPDH was purified for crystallography by a modification of the method of Suzuki and Harris[15], and for sequence work according to Hocking and Harris[19]. The sequence of the enzyme was determined largely (85%) by the automated Edman degradation technique with the aid of both spinning-cup and solid-phase sequencers (see legend to Fig. 1). The preparation, isolation and sequence determination of fragments of the enzyme are described elsewhere[20].

Holo-GPDH (with 4 mol NAD per tetramer) was crystallised by vapour diffusion against 2.4–2.7 M $(NH_4)_2SO_4$. The crystals contain a complete tetramer in each asymmetric unit. Rotation–oscillation data were collected on an Arndt-Wonacott camera[21]. An initial electron density map at high resolution was calculated by isomorphous replacement from two derivatives, and improved by use of the molecular symmetry[22,23].

Molecular structure

The complete amino acid sequence (Fig. 1) is highly homologous with that of the lobster enzyme[1,24]. Of the residues, 51% are identical in the thermophile and lobster enzymes, as compared with 70% identity between lobster and pig, and 68% between the lobster and yeast enzyme[1]. The essential cysteine 149 (ref. 25) is retained in a stretch of conserved residues. The number of arginines is increased from nine to 15 and the number of cysteines reduced from five to two. Lysine 183 which takes part in an acyl transfer reaction[26,27] in muscle apo-GPDH (NAD-free) is replaced by arginine in the thermophile enzyme.

A diagrammatic representation of the whole molecule is shown in Fig. 2. The overall structure of the subunit of the thermophile holo-GPDH is closely similar to that described for lobster GPDH, consisting of the two folding domains shown in Fig. 3. The first domain, residues 0–148, contains most of the residues involved in NAD binding. The secondary and tertiary structural features of the lobster enzyme are reproduced quite accurately. Similar folding domains have been found in other nucleotide binding proteins[28-31]. Side chains implicated in catalysis are contained in the second domain, the region comprising residues 149–333. In this domain the interaction of the β-sheet regions, β_5 with β_6, and β_7 with β_8, is different from that of the lobster enzyme and gives rise to a modified hydrogen-bonding pattern. This change results in the burying of aspartic acid 293 which has significance for thermostability (see below).

An important feature of this domain is an irregular S-shaped loop of polypeptide chain (residues 178–201) which is in contact with the NAD and with the S loop and the α_c helix of the subunit related by the dyad axis marked R (see Fig. 2). In addition, this S-loop interacts with several amino acids across the P axis.

The sequence of the S loop is highly conserved in the lobster, pig and yeast enzymes, whereas the homology between the thermophile and mesophile S-loop sequences is only average. This is surprising since the S loops form the core of the tetramer, and most of their residues are internal, and essential to the interaction across the R axis. Many hydrogen-bonding groups in the main chain of the S loop seem to be unsatisfied in both the lobster and thermophile enzymes. Conserved residues are similarly oriented in both structures. The conformation of the

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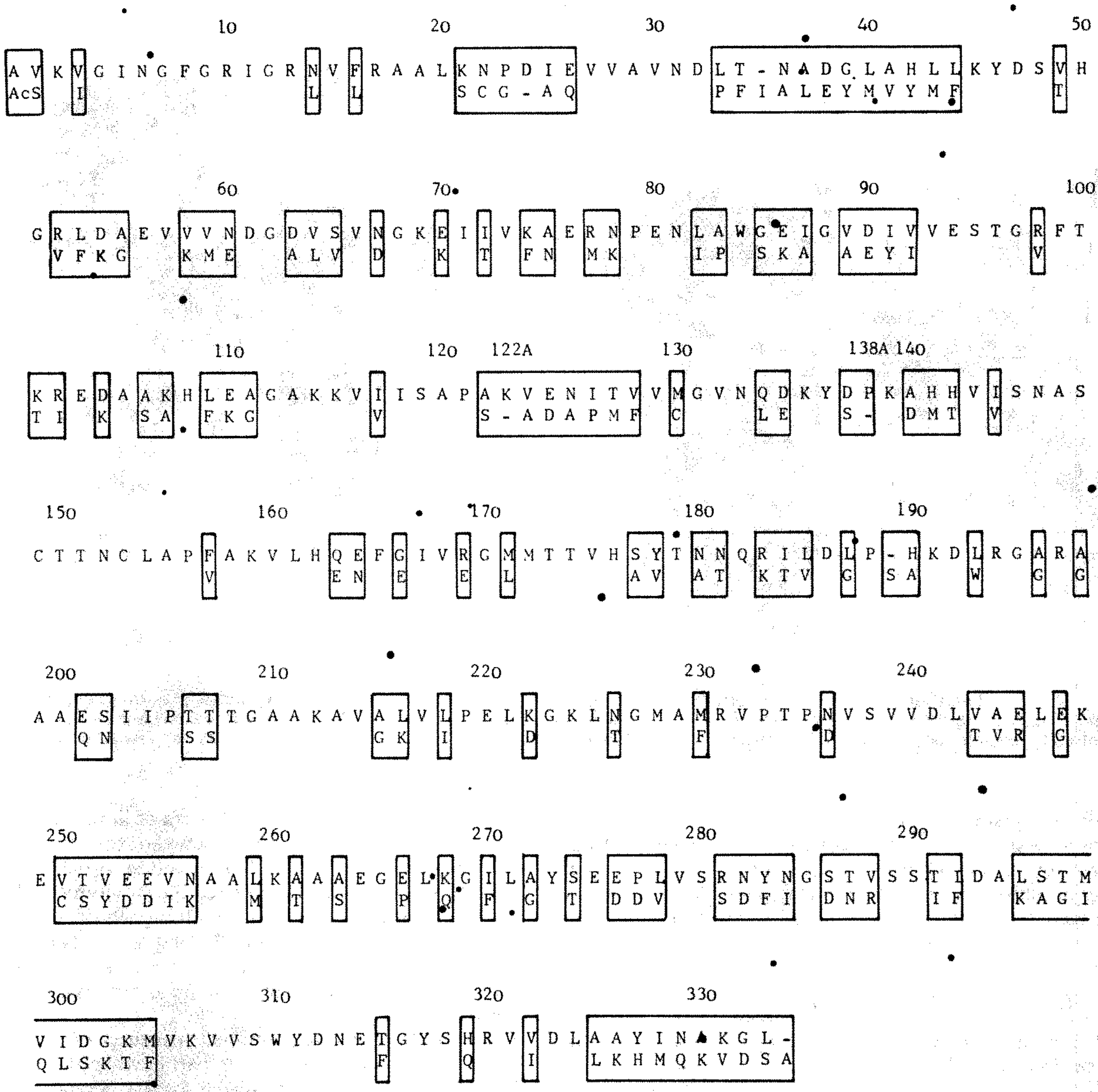

10 20 30 40 50
A V K V G I N G F G R I G R N V F R A A L K N P D I E V V A V N D L T - N A D G L A H L L K Y D S V H
AcS I L L S C G - A Q P F I A L E Y M V Y M F T
60 70 80 90 100
G R L D A E V V V N D G D V S V N G K E I I V K A E R N P E N L A W G E I G V D I V V E S T G R F T
V F K G K M E A L V D K T F N M K I P S K A A E Y I V
110 120 122A 130 138A 140
K R E D A A K H L E A G A K K V I I S A P A K V E N I T V V M G V N Q D K Y D P K A H H V I S N A S
T I K S A F K G V S - A D A P M F C L E S - D M T V
150 160 170 180 190
C T T N C L A P F A K V L H Q E F G I V R G M M T T V H S Y T N N Q R I L D L P - H K D L R G A R A
V E N E E L A V A T K T V G S A W G G
200 210 220 230 240
A A E S I I P T T T G A A K A V A L V L P E L K G K L N G M A M R V P T P N V S V V D L V A E L E K
Q N S S G K I D T F D T V R G
250 260 270 280 290
E V T V E E V N A A L K A A A E G E L K G I L A Y S E E P L V S R N Y N G S T V S S T I D A L S T M
C S Y D D I K M T S P Q F G T D D V S D F I D N R I F K A G I
300 310 320 330
V I D G K M V K V V S W Y D N E T G Y S H R V V D L A A Y I N A K G L -
Q L S K T F F Q I L K H M Q K V D S A

Fig. 1 The primary structure of GPDH from *B. stearothermophilus* (upper) and lobster. The sequence of the thermophile enzyme was obtained by automated sequence analysis of fragments obtained from S(2 — ^{14}C)-carboxymethylated enzyme with cyanogen bromide, trypsin digestion of citraconylated protein and cleavage of succinylated protein with hydroxylamine[53] and with BNPS-skatole[54]. The remainder of the sequence was obtained by the dansyl-Edman degradation[55] of peptides from sub-digests of cyanogen bromide fragments with trypsin and staphylococcal protease[56]. The sequence of the lobster enzyme is from ref. 24. The residues of the enzymes are numbered to maximise homology between the sequences. Hence the N-terminal residue of the thermophile GPDH is numbered 0; deletions are introduced at residues 35 and 189 and insertions at 122 A and 138 A of the *B. stearothermophilus* protein.

S loop appears flexible, but is maintained by the packing of side chains and by interactions with neighbouring subunits made through the contacts across the P and R axes.

Those residues which form the extensive interface between subunits related by the P axis are highly conserved. The Q-axis interface involves few residues but these retain the same specific interactions as in the lobster enzyme[32].

Active site

The NAD binding region is shown in Fig. 4. The coenzyme is bound as in lobster GPDH, differences being confined to a few residues interacting with the adenosine portion of the coenzyme. All the residues implicated in substrate binding and catalysis are conserved except asparagine 181. We observe the same two strong electron-dense features which have been interpreted as bound anions and are probably the sites of the phosphate groups of the substrates during the catalytic reaction (Fig. 4).

According to Trentham's mechanism[33,34] (Fig. 5), glyceraldehyde 3-phosphate reacts with the enzyme to form a hemithioacetal (II), which is converted to an acyl-enzyme (III) with the transfer of a hydride ion to the nicotinamide C4. After NADH is replaced at the active site by NAD (IV) the acyl enzyme is phosphorylysed to give the product 1,3-diphosphoglycerate (V). In agreement with the results of Moras *et al*[12], a model of the hemithioacetal built into the active site with the substrate chain in an extended conformation points the hydride atom of C1 towards C4 of the nicotinamide and puts the 3-phosphate in the 'substrate' anion binding site (P_s in Fig. 4). The C1 oxygen is thought to be protonated by the N^6 of histidine 176, and the C2 hydroxyl of the D isomer can hydrogen bond to the hydroxyl of serine 148. The L isomer which is not a substrate, cannot bind in this orientation because its C2 hydroxyl would interfere with the imidazole ring of histidine 176. These conclusions agree broadly with those reached for the lobster enzyme.

Three amino acid side chains play an important part in the 'substrate' anion site (P_s). Two of these (threonine 179, asparagine 181) are in the S loop and the third (arginine 231) is surrounded by other side chains of the S loop. The S loop thus connects directly the NAD binding site of one subunit to the catalytic site of the R-axis related subunit.

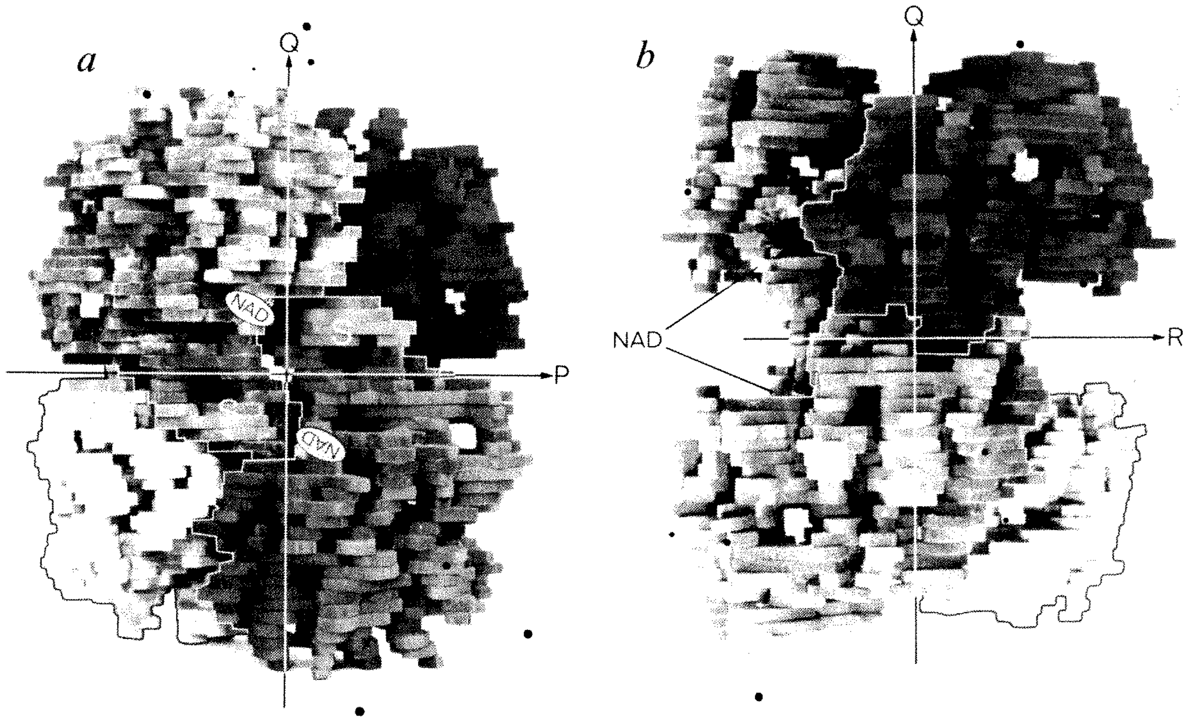


Fig. 2 The four subunits are related by three orthogonal twofold symmetry axes, designated the P, Q and R axes in accordance with the nomenclature adopted[31] for lactate dehydrogenase and lobster GPDH. The four NAD molecules bind to the enzyme close together near the centre of the molecule. *a*, The enzyme tetramer viewed down the R-axis; the S-loop regions are labelled 'S'. *b*, Viewed down the P axis.

NADH is replaced by NAD (IV, Fig. 5); this accelerates phosphorolysis and promotes specificity for phosphate instead of water as the acyl acceptor[35]. Model building shows that the attacking phosphate in the transition state (V) can be stabilised by hydrogen bonds with serine 148 and threonine 150, the amide nitrogens of cysteine 149 and threonine 150, and the C2 hydroxyl of the substrate. The C1 oxygen can be protonated by histidine 176. The 3-phosphate remains in the phosphate site P_s. The close proximity of the sulphur of cysteine 149 to the positively charged nicotinamide C4 would explain the acceleration of the reaction by NAD.

'Racker' band

The characteristic absorption band at λ_{max} 360 nm in holo-GPDH, the 'Racker' band[36], has been explained by the close association of the sulphydryl of cysteine 149 and the nicotinamide ring giving rise to a charge transfer complex[12].

We find that the addition of Hg^{2+} to holo-GPDH abolishes the Racker band, but unlike carboxymethylation, it does not weaken the binding of NAD to crystals. The crystal structure shows that mercury binds behind the active site between the SH groups of cysteines 149 and 153, rotating the side chain of cysteine 149 so as to remove the interaction between the SH and the nicotinamide, but it produces no other structural changes. It follows that this interaction can make only a small contribution to the NAD binding energy. The destruction of the Racker band by mercury without any other changes in the environment of the nicotinamide supports the assignment of the band to a charge transfer complex[37,38]. The distance of at least 3 Å between the SH of cysteine 149 and the nicotinamide ring rules out a covalent interaction[36].

Thermostability

B. stearothermophilus GPDH retains structural integrity and enzymatic activity at temperatures up to 60–70 °C (refs 39, 40), while the *Thermus aquaticus* enzyme[41] remains stable and active to 95 °C. The additional stabilisation energy needed to produce these dramatically increased thermostabilities amounts to only 5–10 kcal. Attempts to identify the source of increased thermostability by relating amino-acid sequences of the thermophile GPDHs to molecular models[41,42] were of limited success owing to the difficulty of distinguishing between sequence changes that are due to phylogeny from those involved in thermal stabilisation. Perutz and Raidt[43] compared the structures and sequence of ferredoxin from a mesophile with the sequences of the same enzyme from various thermophiles and found that their greater heat stability arises mainly from external salt bridges linking residues near the amino terminus to others near the carboxy terminus.

Now that the three-dimensional structures of a thermophilic enzyme and its mesophile counterpart have been independently determined it is possible to search for the stereochemical basis of the extra thermal stability. At the level of accuracy so far attained for the structures, the areas of hydrocarbon surface forming buried contacts within the individual subunits are not significantly different in the two enzymes. Changes in the internal hydrophobic residues between lobster and *B. stearothermophilus* are complementary and are accommodated without structural change. While the P-axis interfaces are essentially the same in both proteins, the hydrophobic interactions at the R-axis interfaces differ especially among residues 184–187. Several aromatic residues are in contact at the edge of this interface in lobster but not in *B. stearothermophilus*. Three tyrosines on the α_c-helix stack with a tryptophan in the S loop of the R-axis related subunit, giving rise to hydrophobic interactions between subunits, not present in the thermophile enzyme, which would indicate greater stability for this region of the lobster enzyme. Contrary to previous speculation, we find the core of the thermophilic enzyme to be no more hydrophobic than that of the mesophilic enzyme.

Although there is a close correspondence between the two structures, a comparative enumeration of hydrogen bonds cannot be made accurately, as their presence can only be inferred from the disposition of hydrogen-bonding groups which are very sensitive to detailed interpretation.

The *B. stearothermophilus* enzyme contains more arginines and two of these extra arginines form salt bridges across subunit interfaces. Arginine 194 forms a buried salt bridge with aspartic acid 293 from the P-axis related subunit. Due to the different conformation of the β-sheet in the catalytic domain this particular aspartate is buried in the thermophile while it is accessible to solvent in the lobster enzyme structure. This salt bridge would not have been predicted simply by relating the sequence of the thermophile enzyme to the molecular model of the lobster enzyme. In addition, arginine 281 of one subunit and arginine 281 from the Q-axis related subunit form a double salt-bridge with glutamic acid 201 from both the R- and P-axis related subunits. These interactions are shielded from solvent and bring the S loops from two subunits into contact with the other two subunits. These additional inter-subunit salt-bridges evidently stabilise the tetrameric structure of *B. stearothermophilus* GPDH, but we would expect that the overall stability of a thermophilic multisubunit enzyme also requires stabilisation of the tertiary structure of the monomer[41]. The detailed structural

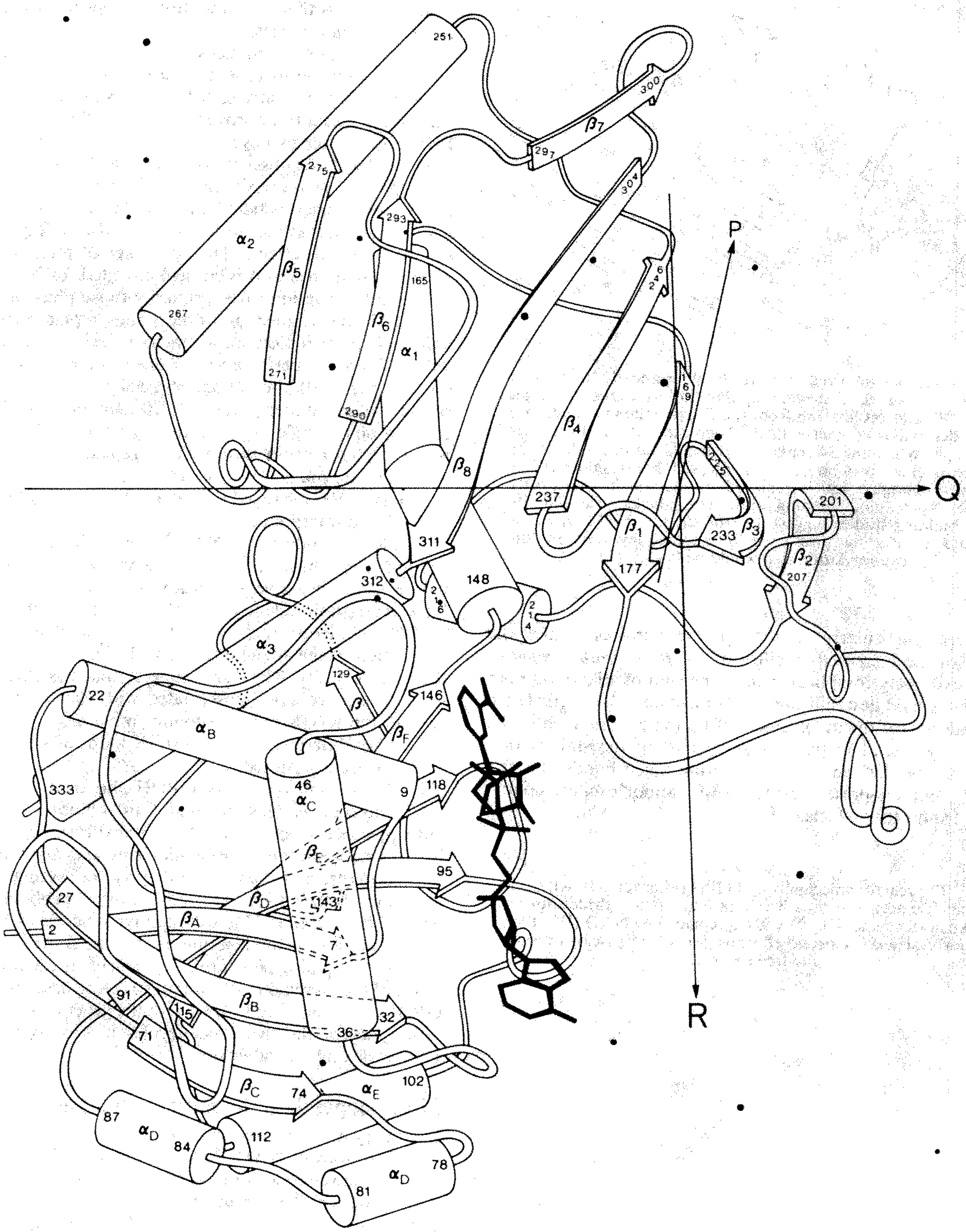


Fig. 3 A folding diagram of one subunit of *B. stearothermophilus* GPDH. The nomenclature for the sheets and helices of the first domain is that used for lactate dehydrogenase and lobster GPDH (ref. 31). The folding in the first domain (residues 0–149) is a β–α–β pattern with a central sheet covered on both sides by helices. In the second domain the extensive anti-parallel β-sheet region forms a subunit interface with the equivalent region of the P-axis related subunit. The S loop (residues 178-201) forms the intersubunit contact with the S loop and α_C helix of the subunit generated by the R axis. The carboxy-terminal helix, α_3, fits into a groove in the first domain. The secondary structure closely resembles that of lobster GPDH except where there are insertions or deletions in external loop regions between segments of secondary structure.

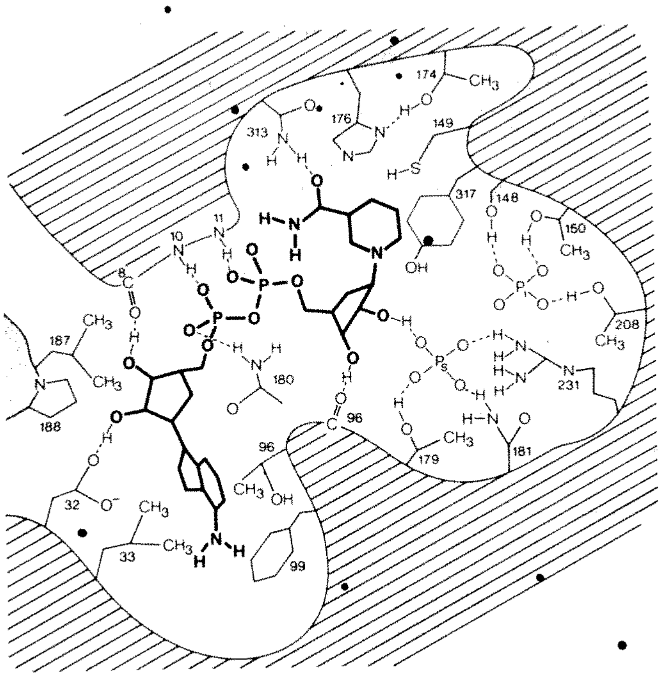
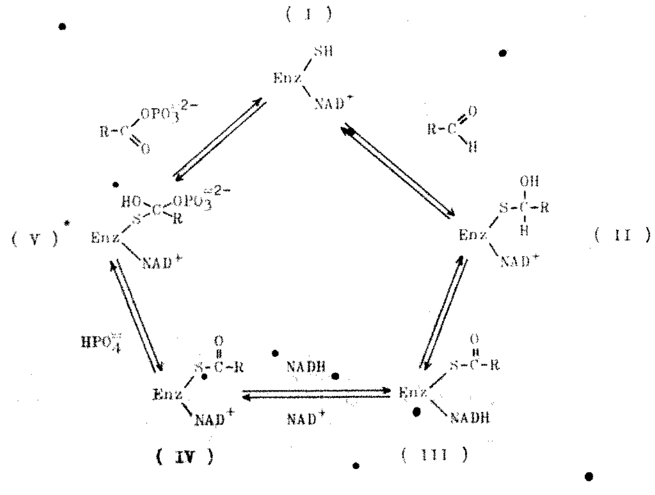


Fig. 4 Residues involved in NAD binding and the two anion binding sites in *B. stearothermophilus* holo-GPDH. Residues leucine 187 and proline 188 from the R-axis related subunit are in van der Waals contact with the adenosine ribose. In lobster GPDH phenylalanine 34 replaces leucine 33 on one side of the adenine pocket: proline 188 is conserved but residue 187 is glycine. Asparagine 180 is replaced by alanine in the lobster enzyme. The anion binding sites are labelled P_s and P_i. Model building studies suggest that the site for the 3-PO_4 of the substrate is P_s; the inorganic phosphate involved in the phosphorylation step would be P_i (ref. 12).

differences, particularly at the enzyme surfaces, make the analysis of the increased stability of the *B. stearothermophilus* enzyme extremely complicated since the sum of a large number of stabilising and destabilising effects must be considered. There is no evidence for the involvement of bound cations such as the calcium ions found in thermolysin[44]. Part of the stabilisation energy may be derived from surface hydrogen bonds and salt bridges[43], but on present evidence we cannot exclude contributions from other effects.

Fig. 5 The reaction mechanism of GPD (after ref. 33). After the enzyme (I) reacts to form a hemithiacetal (II) a hydride transfer gives an acylenzyme (III). NADH is replaced by NAD (IV), then phophorolysis via the proposed transition site (V) yields product and free enzyme.



Molecular symmetry

Folding of the main chain is very similar in the lobster and *B. stearothermophilus* enzymes but there are differences in the interpretation of the molecular structures. Although the subunits of the fully ligated form of GPDH from *B. stearothermophilus* are not related by crystal symmetry, they are nevertheless related by 222 symmetry. The positions and occupancies of five heavy atom sites show the 222 symmetry to be accurate. No structural differences between the four subunits had to be invoked to fit them to the four independently determined electron density images.

An averaged electron density map was not used for interpretation. Starting from a map based on isomorphous replacement with two derivatives, the non-crystallographic symmetry was incorporated to improve the phase angles by the method of Bricogne[23]. After two cycles of this procedure a final map was calculated with the observed structure amplitudes and the phases from the final phase recombination[45]. The map obtained by this procedure is much better than it would have been after averaging, which reduces the noise level but also smears out detail, especially of side chains. The advantage of phase combination over averaging has also been demonstrated for hexokinase[46].

This technique improves primarily those phase angles which are poorly determined by isomorphous replacement, but it is valid only if the molecular symmetry is accurate. For reflections with a figure of merit, $m > 0.7$ from isomorphous replacement (56% of the total), the average phase change after incorporation of molecular symmetry was only 20°. In contrast, the standard error in phase angle expected for $m = 0.8$ is 37°, which shows that the phases from isomorphous replacement are consistent with accurate molecular symmetry.

Moras *et al.*[12] averaged their map of lobster GPDH about the Q dyad and interpreted the map in terms of a non-symmetrical molecule, concluding that NAD is bound differently to different subunits. They have advanced this asymmetry as a partial explanation of the negative cooperativity of NAD binding. In their interpretation the adenine ring of NAD of one subunit is rotated by 180° relative to that of the R-axis related subunit, and the interaction of the adenine ribose with the protein is different in the two subunits. They also suggest that cysteine 149 in one subunit is more closely associated with the nicotinamide ring and, in the R-axis related subunit, more closely with histidine 176. Other differences between the S loops of symmetry related subunits are also noted.

In *B. stearothermophilus* GPDH the conformation of the NAD molecule is the same in all four subunits. The four NAD binding sites are equivalent. The sulphydryl of cysteine 149 lies halfway between the nicotinamide ring and histidine 176 in all four subunits, and there is no density connecting cysteine 149 and the nicotinamide. Histidine 176 is probably hydrogen bonded to threonine 174, although tyrosine 311 is close by, and the orientation of the histidine ring is the same in all subunits. The anion binding sites are also present in all four subunits. None of the asymmetry of subunit structure described for lobster GPDH has been found. In view of the functional and structural similarities of the two holoenzymes it is unlikely that one is symmetric and the other not.

NAD-dependent structural changes

Symmetry of the enzyme is maintained either in saturating concentrations of NAD, or in the absence of NAD. In crystals grown in lower concentrations of NAD, or soaked in NAD-free mother liquor, 0.5–1.5 moles of NAD are lost and structural changes in the protein appear in difference maps against holo-GPDH (ref. 48). The largest changes occur in the nucleotide binding domain of the subunit which has lost its NAD. The conformation of this domain changes towards that observed in a 6-Å map of apo-GPDH (ref. 47).

The large movement of the coenzyme-binding domain on loss of NAD to form apo-GPDH gives rise to an expansion of the molecule. An increase in molecular volume upon loss of NAD has also been detected by sedimentation equilibrium studies[48,50], and by small-angle X-ray scattering[51,52].

The negative cooperativity of NAD binding could be the result of a change in packing of parts of the protein chain, especially of the residues in the S-loop. In holo-GPDH this segment of 20 residues is devoid of secondary structure and most of its main chain is inaccessible to solvent. Expansion of the molecule in apo-GPDH opens this region and might make the S-loop more flexible. The following chemical evidence supports this. If muscle apo-GPDH is acylated, and then incubated at *p*H 8.5, the acyl group is transferred specifically from cysteine 149 to N^ε of lysine 183 (refs. 26, 27). (Residue 183 is arginine in the bacterial enzyme, but the position of the arginine side chain is the same as that of the lysine 183 in lobster GPDH.) In holo-GPDH this transfer does not happen because the N^ε is 10 Å from the sulphydryl of cysteine 149. The occurrence of the reaction in apo-GPDH only, is indicative of the greater flexibility of the S-loop.

The S-loop has many of the properties expected of a structure which transmits a structural change from one subunit to another in a cooperative system. It interacts with two adjacent subunits (including a direct coenzyme contact), it is shielded from solvent at least in holo-GPDH and lacks strong secondary and tertiary interactions. In addition, there is evidence that its structure in the apoenzyme is quite different from that in the holoenzyme.

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letters to nature

A better way of searching for black-hole explosions?

BLACK holes of $\lesssim 10^{15}$ g evaporate in $\sim 10^{10}$ yr, and eventually annihiliate into a burst of energetic photons and particles[1]. To test this theoretical prediction would be extraordinarily significant for quantum and gravitational physics. There could be $\sim 10^{23}$ 'miniholes' within our Galaxy[2–4]; on the other hand, there may not be any at all. But even if they exist in profusion, how best could we detect black-hole explosions? Attention has been focused on the problem of directly detecting the γ rays, but the prospects seem bleak[3]. The possibility that the particles ejected in the explosion may generate conspicuous effects has hitherto been overlooked. I argue here that collective interaction of electrons and positrons with an ambient interstellar magnetic field can (on some specific assumptions) generate radio bursts powerful enough to be detected from anywhere in our Galaxy, or even beyond. This opens up a more promising perspective on the search.

A conducting sphere, expanding into a uniform magnetic field, will develop surface currents which prevent the field from penetrating it. If the shell expands relativistically, with constant Lorentz factor $\gamma \gg 1$, out to a radius r_{max}, then the work done against the field is

$$\sim \gamma^2 \times (\text{energy of swept-up field}) \qquad (1)$$

(Note the factor γ^2, which occurs just as it would if the sphere were reflecting electromagnetic radiation.) A distant observer would see the part of the shell moving at angles $\lesssim \gamma^{-1}$ to the line of sight blue-shifted by $\sim \gamma$; he sees a surface current on this part of the shell increasing $\propto t^2$ for a time $\sim r_{\text{max}}/c\gamma^2$. The energy (1) therefore appears as an electromagnetic pulse with characteristic wavelength

$$\lambda \simeq r_{\text{max}}/\gamma^2 \qquad (2)$$

We now investigate how this classical electromagnetic phenomenon might apply to the type of 'fireball' resulting from an exploding black hole.

Suppose that, when the mass of an evaporating minihole has fallen to M_{crit} g, it vomits forth its remaining energy in an essentially instantaneous burst of $\sim 10^{31}\, M_{\mathrm{crit}}$ erg. In the Hagedorn theory, $M_{\mathrm{crit}} \simeq 10^{14}$ g; but on other theories of elementary particles it would be lower. Very near the hole, the energy would be distributed between a variety of exotic species; but we assume that these quickly decay, and that, by the time the ejecta have expanded to macroscopic radii, a substantial fraction (say $\sim 50\%$) of the energy is in a 'fireball' of electron–positron pairs[15]. From the theoretical relation between temperature and mass, we expect this fireball to expand with $\gamma_f = (M_{\mathrm{crit}}/2\times10^{16}\ \mathrm{g})^{-1}$.

For illustrative purposes, we consider fireballs with energy $\varepsilon_f \simeq 10^{37}\ \gamma_f^{-1}$ erg in electron–positron pairs. The number of pairs is then $N_f \simeq 10^{43}\ \gamma_f^{-2}$.

We must then address these questions:

(i) How large a radius can the fireball attain before the energy in the swept-up field decelerates it?

(ii) To what extent is it a good enough conductor to sweep up the field?

(iii) Would the fireball be braked by sweeping up external plasma as well as magnetic flux?

Suppose that the uniform external magnetic field is $B \simeq 5\times10^{-6}\ b$ G (where b would be ~ 1 for a typical interstellar field with energy density $\sim 10^{-12}$ erg cm^{-3}). If the fireball behaved as a perfect conductor, it would be significantly braked when it had swept up a magnetic field energy of $\sim \varepsilon_f/\gamma^2{}_f$. Thus we have, on energetic grounds, an upper limit to r of

$$r_{\mathrm{en}} \simeq (10^{16}/\gamma_f) b^{-2/3}\ \mathrm{cm} \qquad (3)$$

Quite apart from this limit, the fireball cannot behave like a perfect conductor and expel the magnetic field (without being decelerated) unless the charge density is sufficient to carry the currents. This requires a surface density $\sim 10^3 \gamma_f\, b$ charges per cm^2. The conductivity condition thus breaks down at a radius r_{cond} where $N_f/2\pi r^2$ falls to this value. One finds

$$r_{\mathrm{cond}} \simeq (4\times10^{19}/\gamma_f^{3/2}) b^{-1/2}\ \mathrm{cm} \qquad (4)$$

The appropriate value of $r_{\max}$ to use in (1) and (2) is the smaller of r_{en} and r_{cond}. For $\gamma_f < 10^7$, r_{cond} exceeds r_{en} (that is, the conductivity is good at all relevant times); so a fraction ~ 1 of the total fireball energy then emerges as electromagnetic waves of characteristic wavelength $\lambda \simeq r_{\mathrm{en}}/\gamma_f^2$. This means that, for $\gamma_f \simeq 10^5$, $\sim 10^{32}$ erg emerges at $\lambda \gtrsim 10\, b^{-\frac{2}{3}}$ cm; and for $\gamma_f \simeq 10^7$, $\sim 10^{30}$ erg emerges at $\sim 10^4\, b^{-\frac{2}{3}}$ Å.

Such a radio burst would be readily detectable anywhere in our Galaxy, or even beyond[6,7], and the optical flash from a $\gamma_f \simeq 10^7$ fireball could be detected $\gtrsim 1$ kpc away (J. V. Jelley *et al.*, to be published). We must, however, check the validity of our assumption (question (iii) above) that the fireball is not braked by external plasma before attaining a radius r_{en}. External particles can be swept up, yielding a relativistic blast wave, only if the gyroradius of a particle with Lorentz factor γ_f in a field γ_f B is $< r/\gamma_f$. This requires

$$r \gtrsim 4\times10^8\, b^{-1}\, \gamma_f \qquad (5)$$

In the cases $\gamma_f \simeq 10^5$ and 10^7 cited above, $r_{\max}$ ($= r_{\mathrm{en}}$) does not satisfy (5) for $b \lesssim 1$, so we are justified in assuming that the fireball transfers its momentum to the field rather than the ambient particles. (Arguments analogous to those used in deriving (4) and (5) show that the weak steady efflux preceding the final explosion cannot create a field-free heliosphere-type cavity around a minihole. This vindicates our assumption that the fireball impacts directly on to a typical ambient magnetic field).

In the 'extreme Hagedorn' case ($\gamma_f \simeq 300$), where the earlier argument would have yielded a strong ultra-low-frequency pulse that in any case would not propagate freely, (5) is fulfilled, so we would expect a relativistic blast wave with a swept-up shell. There is then no chance of getting efficient coherent radiation, the energy going instead into random relativistic particle motions. The field strength in the shell would be $\sim 5\times10^{-3}\ b$ G, and relativistic electrons with $\gamma_f \simeq 300$ would lose only 10^{-10} of their kinetic energy while the shell expanded. If a Hagedorn-type explosion occurred on a timescale Δt sudden enough to cause a coherent radio pulse, then most of the pairs would in any case have annihilated into photons. (The expansion timescale, in the comoving frame, when the fireball temperature falls to ~ 0.5 MeV is $\propto \gamma_f^{-2}(\Delta t)^{1/2}$, and our assumption that there is no time for annihilation to occur is self-consistent for $\gamma_f \gtrsim 10^5$.)

It is thus the case $\gamma_f \gg 10^3$ which—even though it entails a lower ε_f—offers the greatest promise of radio (or optical) detection. The pulse-production mechanism requires that the final outburst be 'instantaneous' in the sense that its timescale is $\lesssim \lambda/c$. This requirement is perhaps not met by quark theories, unless a lot of extra species occur above some energy $\gtrsim 100$ GeV. Because of this sensitivity to the uncertain particle physics, the absence of frequent strong radio pulses reaching the Earth cannot be used to improve the present upper limits on the number of miniholes in the Galaxy[2–4]. On the other hand, linearly polarised radio-frequency pulses (rather than γ-ray pulses) may well be much the most conspicuous manifestations of black-hole explosions, because radio techniques are exceedingly sensitive[6,7]. To dramatise the potential advantage, the γ rays from a fireball with $\gamma_f \simeq 10^5$ would only be registered by a detector of effective area 100 cm^2 if the event occurred within 10^{-2} pc; but even a crude non-directional antenna would detect the corresponding radio pulse at distances $\sim 10^4$ pc. And an Arecibo-type telescope could detect such pulses—each triggered by a single entity of subnuclear size—from as far away as the Andromeda galaxy!

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Is Cas A illuminated by a binary system?

I REPORT here indications that the supernova remnant Cas A (and probably many others) contains a neutron star at its centre, in close orbit around a 'normal' star. The indications are of three types: of evolutionary, statistical and indirect character, arguing that the high observed magnetic field strengths and the steepening synchrotron spectrum cannot be explained by stochastic processes.

Supernova explosions are commonly believed to derive their energy from the collapse of the (degenerate) core of a formerly massive late star. The conversion of gravitational energy into the kinetic energy of radial motion could be achieved through a magnetic spring[1–3] rather than a combination of neutrino[4] and thermal pressures. The mass of the collapsing core is expected to be near the Chandrasekhar mass ($\approx 1.4 M_\odot$), because this is the minimum mass for gravitational instability according to stellar evolution theory, and because all the overlying material is ejected by the action of the magnetic spring. The core thus turns into a stable neutron star. Mass determinations[5] of neutron stars in binary systems confirm this picture, which might apply to supernovae of all types (I, II,...), their differences being due to the density of the surrounding medium which controls the mass in the envelope.

Most if not all of the massive progenitor stars of supernovae occur in binary (or multiple) associations. During the explosion, a binary system gets disrupted if the ejected mass is bigger than the remaining mass, which can happen in the first or, more likely, in the second event. If in the first event, we obtain (eventually) two pulsars; if in the second, mass flow from the normal star to the neutron star can give rise to a temporary X-ray source, and eventually to one or two pulsars (depending on the spin rate of the first neutron star). The measured peculiar velocities[6] of 12 pulsars suggest that several of them received two kicks in the past, and hence result from binary systems containing a neutron star.

We therefore expect a large number of supernova explosions to leave a binary neutron star at their centre. What will such a system look like? The young neutron star should act as a strong pulsar, with a relativistic wind of power $L \approx 10^{39}$ erg s^{-1} $\Omega_{2.5}^{4}$ (modelled after the Crab). A fraction of this power will heat the atmosphere of the companion star until enough plasma is evaporated for the formation of a common envelope. The time scale t for evaporating a small fraction α of the companion star's mass M is of the order (ignoring reradiation)

$$t = \alpha GM^2a^2/R^3L \approx 30y\alpha_{-3}a_{12}^2$$

where a is binary separation and R the stellar radius. For close binaries, a small evaporated mass fraction α should be sufficient to build up an atmospheric pressure which overcomes the pulsar wind pressure, first at mid-separation, and later at all distances outside a small cavity around the neutron star, so that a common envelope forms, and the pulsar is quenched. Instead of driving a relativistic wind, the rotating magnetised neutron star will henceforth accelerate the wind plasma of the companion star which impinges on its magnetosphere (through magnetospheric friction), and produce cosmic rays[7]. It will no longer act as a pulsar. Cyg X-3 could be a system in transition between the pulsar stage and the cosmic-ray stage; whereas Cas A is suggested to have reached the latter.

To obtain an independent estimate of the duration t of the pulsar stage, and to assure consistency of the whole model, we consider statistics. The present birth rate of supernovae in the Galaxy has been estimated[8] to lie between 10^{-1} and 10^{-2} yr^{-1}. Allowing for an enhanced activity throughout an early phase of the Galaxy, the total number of supernova explosions since formation should be 10^8 to $\gtrsim 10^9$. Unless neutron stars can be formed in a peaceful way[9,10]—which is not very likely even when magnetic fields are ignored—this number must not be smaller than the total number of neutron stars in the Galaxy. Neutron stars occur singly, as pulsars, and in binaries, visible during the X-ray stage. Extrapolation of the known data[11–13] on pulsars leads to a present total number between 10^4 and 7×10^5, depending on the distance scale, and upon the amount of beaming into a reduced spherical angle. Pulsar lifetimes peak near 3×10^6 yr so that there should be (at least) a total of 2×10^7 to 2×10^9 single neutron stars (= dead or alive pulsars) in the Galaxy. (Single neutron stars ejected as slow rotators from binary systems are not included.) This suggests a one-to-one correspondence between supernova explosions, and neutron star formations; Cas A may not form an exception.

Next, a single pulsar has not been seen in orbit around a normal star, although there should be[14,15] over 10^4 such systems in the Galaxy (inferred from the number of X-ray sources, or from the number of pulsars.) This implies that the pulsar stage t must be $\lesssim 10^{-4}$ times shorter than the lifetime of a massive star, that is, shorter than some 5×10^2 yr.

Cas A has an estimated age[16] of 3×10^2 yr, and a radius of 5 light yr. It does not contain a pulsar: even if the pulses were beamed away from us, we should see strong optical emission from the inner edge of the nebula, like the wisps in the Crab, or at least a strong compact X-ray source. Also, pulsar peculiar velocities[17] are rarely higher than 10^{-3} c so that a pulsar could not have escaped from the nebula. For these reasons, Cas A has been held to be a remnant without a central source, and has been treated[18] as a hot cloud expanding due to a powerful explosion in the past. Such a detonation wave becomes unstable, near its outer edge, towards convective motion and mixing with the interstellar plasma. From his numerical models, Gull[18] estimates a conversion of 2 or 3% of the total expansion energy into convection, which is held responsible for driving a dynamo that enlarges the interstellar magnetic field by a factor of $\approx 10^2$, to arrive at the inferred equipartition field strength of 5×10^{-4} G in the outer parts of the remnant. In most of the compact knots, the minimum magnetic field inferred from the radio synchrotron brightness is as high as 3×10^{-3} G. The radio intensity decreases with time, by roughly 1% per yr, but at a higher rate (1.3%) at 85 MHz than at 1.4 GHz (0.9%). Moreover, there is a soft X-ray luminosity of 3×10^{36} erg s^{-1}.

The difficulty faced by this model is the large observed magnetic field, which would require (many) more than seven turns to be performed by the interstellar material; even if there is enough energy available, a dynamo cannot do better than double the seed field during each turnover. With the observed convective velocities $\lesssim 10^{-2}$ c (ref. 18), such a dynamo cannot reach scales beyond 10^{-1} light yr. Similar difficulties arise for the radiating electrons whose synchrotron lifetimes are shorter, at energies above 10^2 GeV, than the lifetime of the remnant, and also much shorter than the replenishment timescale for Fermi acceleration[19].

These difficulties disappear if we assume the existence of a neutron star inside the remnant. This neutron star would have acted as a pulsar for an initial period of the order of some 10^2 yr, during which its extremely relativistic wind injects both electrons and magnetic flux into the supernova shell[20]. When overtaking trailing filaments (protostellar condensations), the frozen-in magnetic field gets hooked up, and forms the magnetic knots—which I interpret as filaments seen edge on. (Such knots do not occur in the Crab nebula, whose symmetry axis is nearly normal to the line-of-sight). For the same reason, the field line geometry between knots is near-radial.

The main reasons against an empty interior of Cas A are therefore, in decreasing order of cogency: (1) The finite scale, strong magnetic fields are difficult to explain, because a dynamo needs time; and relativistic particles are difficult to produce by the more or less ordered (radial) motion of a detonation wave. (2) If the majority of supernova explosions did not result in the birth of a neutron star, we might see more pulsars than have been (violently) born. (3) There is a trend both in late stellar evolution theory, and in astration theory[21] towards assigning an increasingly large probability to neutron star formation in supernova explosions.

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Dirac–Fock one-centre calculations show (114)H_4 to resemble PbH_4

RELATIVISTIC effects have a considerable influence on the chemical behaviour of the heavier elements. Our Dirac–Fock one-centre expansion calculations[1-3] indicate that the X–H bond lengths, R_e, suffer a relativistic contraction of about 5% for PbH_4 (ref. 1) or AuH (ref. 2) and 7% for TlH (ref. 3). If the relativistic effects are included, however, these R_e values mostly agree with experiment to within 2–3%. Relativistic effects also seem to be the main cause of the chemical difference between silver and gold[2] and, possibly, of the dominant monovalency of thallium[3]. A relativistic treatment becomes imperative to predict the chemical behaviour of the superheavy elements. Following evidence for primordial superheavy elements[4], we present here results for the tetrahydride of the element 114 or eka-lead. We believe that such a calculation gives a reasonable estimate for the covalent radius of this element. Although the dominant valency of 114 is expected to be 2, some tetravalent compounds are also expected to be stable[7]. As the latter valency is more convenient for calculations, having closed shells, we here consider (114)H_4.

Our approach here is as in ref. 1. No symmetry functions were used. A finite, homogeneously charged nucleus with $A = 298$ was assumed. The total energy, E_T, was calculated at five internuclear distances and the results were fitted into a third degree polynomial. The results are shown in Table 1, E_T variation in Fig. 1, and orbital energies in Fig. 2. The most striking aspect of the Fig. 2 is that the 6 $d_{5/2}$ shell is slightly above the 7 s. Therefore, a strong participation in chemical bonding is expected from the 6 $d_{5/2}$ and 6 $d_{3/2}$ shells. Similarly, a strong participation of the 5 d orbitals is suspected for AuH (ref. 2). The present values of R_e and k_2, 3.376 and 0.57 AU, respectively, are closely similar to those obtained[1] for PbH_4 using the same approximations, or 3.395 and 0.55 AU, respectively. The experimental k_2 for both $PbH(CH_3)_3$ and $PbH_2(CH_3)_2$ is 0.445 AU (ref. 9).

Table 1 Calculated results for (114)H_4

Bond length R_e	3.376 AU = 1.787 Å
Total energy $E_T(R_e)$	−49712.76 AU
Force constant k_2	0.57 AU
Orbital energies ε*	
7 $p_{3/2}$	−0.25712 AU
7 $p_{1/2}$	−0.43640
7 $s_{1/2}$	−0.80274
6 $d_{5/2}$	−0.77015
6 $d_{3/2}$	−0.93950

*At $R = 3.4$ AU

The covalent radii for elements C to Pb (ref. 6) are listed in the first column of Table 2. Combining them with the experimental R_e values in the second column, we find a covalent radius of 0.31 Å for hydrogen. Subtracting this value from the present theoretical R_e of 1.787 Å we find a covalent radius of 1.48 Å for quadrivalent (114). The difference of 0.04 Å between the R_{cov} for Pb and 114 includes the experimental error for PbH_4. The theoretical R_e differs by 0.01 Å.

As the effects of increasing atomic number, Z, on the bond length are quite different for Groups IB (Au), IIIA (Tl) and IVA (Pb), it is interesting to discuss these differences in terms of the electron configurations. Group IB has an s^1 valence configuration which is also the dominant component of the bonding molecular orbital in AuH. As this atomic orbital contracts strongly[5], due to relativistic effects, the experimental R_e for AuH is 0.09 Å shorter than that for AgH. For Group IIIA the valence configuration is p^1. According to the model in ref. 3, a transition from pσ bonding to $p_{1/2}$ bonding takes place when going from InH to TlH. Therefore the lost directional character makes the chemical bond of TlH weaker. Although the $p_{1/2}$ shell itself contracts, the sum of the two effects makes the R_e for TlH 0.02 Å larger than for InH. For the tetrahydrides of Group IVA, the $p_{3/2}$ and $p_{1/2}$ shells are filled. The average orbital energy $(2\varepsilon_{np_{3/2}} + \varepsilon_{np_{1/2}})/3$ depends only slightly on Z (Fig. 2). The theoretical R_e values increase by 0.03 Å from SnH_4 to PbH_4 and decrease by 0.01 Å from PbH_4 to (114)H_4. No evident interpretation can be given for these small changes.

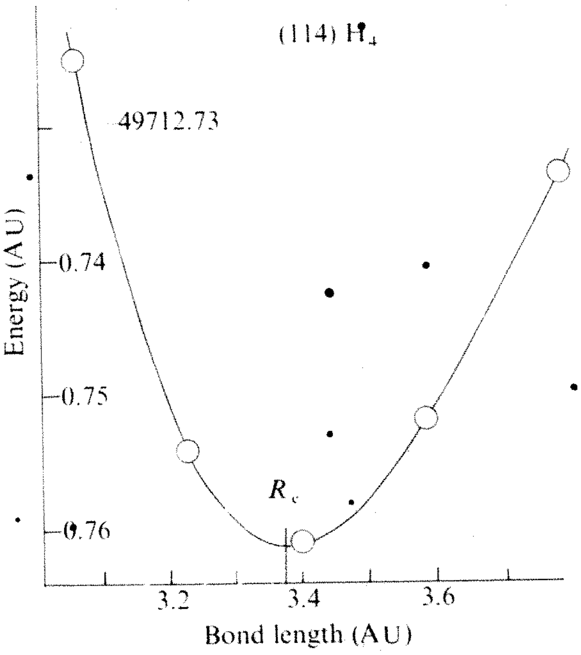

Fig. 1 Molecular total energy as a function of bond length. The curve is the fitted third degree polynomial.

We give here the first calculation of a bond length for a superheavy element. The result for the covalent radius of 114 is about 1.48 Å. Assuming also for metallic 114 the same crystal structure and lattice parameter as for Pb, the assumed atomic weight of 298 would lead to a density of 16.3 g cm^{-3} for the metal 114. The general chemical conclusion is that the covalent radii of 114 and its neighbours are expected to be comparable with,

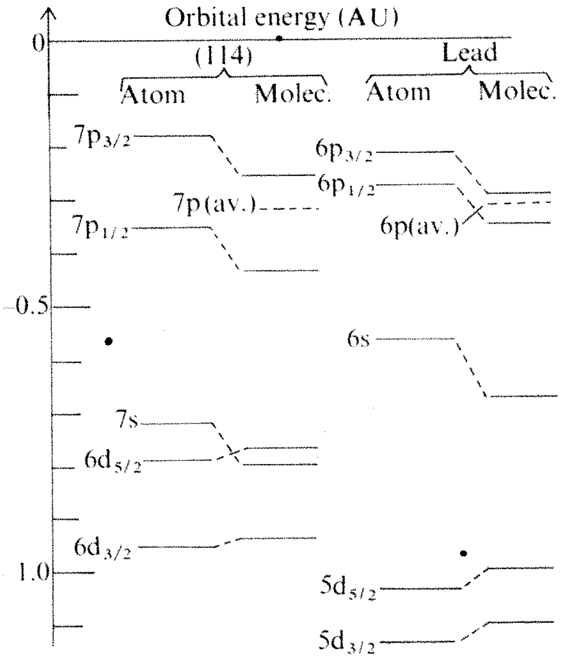

Fig. 2 Orbital energy diagrams for the free atoms of lead and the element 114 (from ref. 5) and for the molecules PbH_4 (from ref. 1, $R = 3.50$) and (114) H_4 (present work, $R = 3.40$).

Table 2 Covalent radii R_{cov} (Å) for the tetravalent elements C to (114), measured X–H distances, $R_{e,exp}$, for the compounds XH_4, and the corresponding distances, $R_{e,calc}$, calculated using R_{cov}.

Element	R_{cov}	$R_{e,exp}$‡	$R_{e,calc}$
C	0.77*	1.093	1.08
Si	1.17*	1.480	1.48
Ge	1.22*	1.527	1.53
Sn	1.40*	1.701	1.71
Pb	1.44*	(1.754)§	1.75
114	1.48†	—	—

*From ref. 6.
†Present work.
‡From ref. 8.
§Deduced from R_e (PbH_4) = R_e (PbH) + R_e (SnH_4) − R_e (SnH).

or slightly shorter than those of lead and its neighbours. The same is true of the force constants. Unfortunately, we cannot draw conclusions about the chemical stabilities as it is known that, for example, PbH has nearly the same R_e and k_2 as SnH but only one half of the dissociation energy[10].

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A melting criterion based on the dilatation dependence of shear moduli

The elastic instability theory of melting, proposed by Max Born[1], held that melting in a crystalline material occurs when one of the elastic shear moduli vanishes, so that the material can no longer sustain an infinitesimal shear stress without flowing. This explains the fluidity of a melt, but fails to account for several other important characteristics of melting. Melting is a transition between two independent phases, while Born's theory is a single-phase theory which contains no distinct description of the melt, and thus fails to account for the discontinuous, first order character of melting. Because it is an essentially homogeneous theory, it does not explain the occurrence of superheating, the metastability of the melt, and the heterogeneous nucleation and growth features of the melting process. Also, subsequent observations of elastic moduli showed that none of the shear moduli is zero or near zero in the solid at the melting point. Because of these defects Born's instability theory currently enjoys little credibility. We believe, however, that correct application of Born's hypothesis leads to a simple modification of his initial criterion for melting which satisfies all the requirements of a physically realistic theory of melting. We show here that the hypothesis does predict a first-order transition between two phases and that the transition has a nucleation barrier, thus dismissing some of the physical objections to the hypothesis. Moreover, the hypothesis applies in the alkali halides, in that in each case one of the shear moduli falls continuously to zero through the melting expansion.

Suppose that one of the elastic shear moduli, μ, decreases monotonically with temperature increase, and that at some critical temperature the shear modulus vanishes or becomes sufficiently small that shear flow could occur by thermal activation. The consequent fluidity which allows each atom access to the entire fluid volume will cause an increase in entropy, due to the communal entropy, which in the case of the alkali halides equals $2R$. The onset of fluidity thus results in a decrease in free energy by $2RT$. In the virtual process of tracing back along the shear modulus curve, initially increasing the free energy by losing the communal entropy but progressively decreasing the elastic internal energy, a point can be located, at which the free energy of the solid equals the free energy of the virtual solid that has a vanishing shear modulus. (The elastic internal energy will be decreased by $> 2RT$, as the virtual process results in an additional decrease in vibrational entropy.) Thermodynamically, it is possible for the solid at the point thus located to transform isothermally to the vanishing shear modulus state. This is the melting point, and the transition will be discontinuous with an accompanying heat absorbtion. We therefore suggest that Born's melting criterion that melting occurs when the shear modulus of the solid vanishes, should be replaced by the following: melting occurs when a solid can transform isothermally to a state of zero shear modulus. The modified theory becomes a two-phase theory in that the solid at the melting point is described as solid at dilatation $\delta_m{}^s$ while the melt at the melting point is described as virtual solid with properties evaluated at $\delta = \delta_m{}^l$. Moreover, there is a nucleation barrier to melting equal to the communal entropy, and consequently the heterogeneous character of melting is also implicated by the hypothesis.

The behaviour of the alkali halides agrees with our proposal. Fig. 1 shows a plot of shear modulus, $\frac{1}{2}(c_{11} - c_{12})$ for the rock salt alkali halides, and c_{44} for CsBr and CsI as a function not of temperature, but of dilatation, that is, the relative increase in volume $\Delta V/V$ from room temperature to the temperature and phase of interest. Our data are obtained from refs 2–5. The last point in the close succession of points on the low dilatation side is the shear modulus at the dilatation, $\delta_m{}^s$ of the solid close to the melting point, while the isolated points for each curve are for $\mu = 0$ at the dilatation, $\delta_m{}^l$ of the liquid at the melting point. Although the shear modulus and the dilatation are both discontinuous with respect to temperature at the melting point, the points for the melt in Fig. 1 lie on the extrapolated curves for the solid, and consequently the shear modulus seems to be a continuous function of dilatation through the melting expansion and falls to zero at the dilatation of the melt. Born's melting hypothesis as modified above thus seems to apply for the alkali halides.

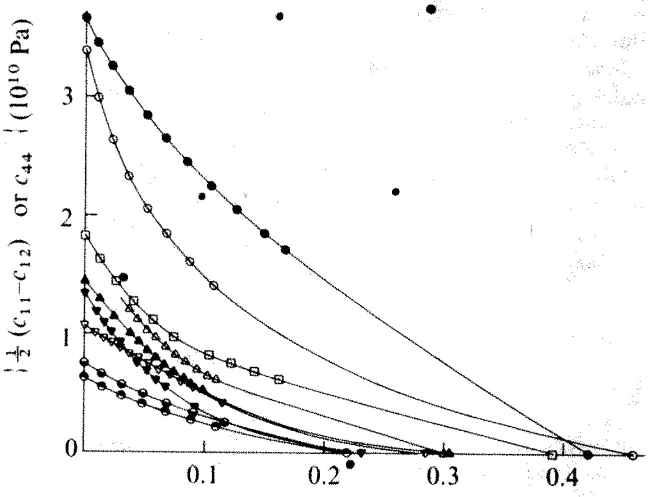

Fig. 1 The shear moduli $\frac{1}{2}(c_{11}-c_{12})$ for some rock salt alkali halides and c_{44} for CsBr and CsI as functions of dilatation, $\delta = \Delta V/V$. ○, LiF; ●, NaF; □, NaCl; △, KCl; ▲, KBr; ▽, KI; ▼, RbI; ◒, CsBr; ◒, CsI.

A fuller account of our work and evidence for the continuity of the bulk modulus with respect to dilatation through the melting expansion are presented elsewhere[6,7].

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Non-radiative energy transfer in polymers

THE study of intra- and intermolecular transfer of energy by non-radiative processes is important in developing an understanding of such phenomena as photosynthesis and photo-stabilisation of polymers. Several theories have been proposed, notable among which is the dipole–dipole mechanism due to Förster[1] and Galanin[2]. Apart from the dependence on distance separating donor and acceptor, the probability of transfer is related to the angle between donor and acceptor moments. For this reason measurements of fluorescence depolarisation can be used to estimate the efficiency of transfer from donor to acceptor. Macromolecules provide interesting potential for studying intra- as well as intermolecular non-radiative transfer. Thus chromophore-bearing polymers such as polystyrene allow the possibility of excitation at one part of the molecule, transfer along the chain from one phenyl unit to another until an energy trap, an excimer site, is reached whereupon the energy is emitted as a photon. The principal emission observed from polystyrene at room temperature is that from excimer. We wish to make a preliminary report on some studies we have made on fluorescence depolarisation using a styrene–phenyl acetylene copolymer as donor, and perylene as acceptor.

The donor–acceptor pair were cast in poly(methyl methacrylate) films. This copolymer has polyene groups along the main chain as well as the pendant phenyl units. It has a much stronger emission spectrum than polystyrene and is thus more suitable for using in energy transfer studies. The results shown in Table 1 indicate that energy is being transferred from donor to acceptor, at the highest concentration of acceptor transfer is almost complete. At this level of concentration of acceptor the process of transfer is long range and the mechanism must be by dipole–dipole interaction. Applying Förster theory[1] to the data yields an R_0 value of 78 nm indicating coupling over relatively long donor–acceptor distances. Column 2 of Table 1 shows that as the efficiency of transfer increases, the extent of depolarisation of the emission from the acceptor also increases, in accord with the predictions of Förster's theory. It is also worth noting that intermolecular energy transfer is occurring involving perylene molecules only (column 4). However, the emission from the macromolecular donor shows no depolarisation which suggests that neither inter- or intramolecular non-radiative energy transfer is taking place.

We also measured the depolarisation of the excimer emission of several samples of pure polystyrene films cast from methylene dichloride solutions and obtained a value of 1.3 ($\lambda_{ex}=264$ nm, $\lambda_{em}=320$ nm). The instrument used to make the measurements was a Perkin Elmer MPF2, using the polarisation accessories provided by the makers. The value obtained for fluorescein was in agreement with the literature value.

Table 1 Depolarisation of styrene/phenyl acetylene copolymer (donor) and perylene (acceptor), suspended in poly(methyl methacrylate)

Acceptor concentration (M)	Efficiency of transfer from donor to acceptor	Depolarisation (1)	Depolarisation (2)	Depolarisation (3)
0	—	—	—	1.8
0.55×10^{-3}	0.40	6.8	3.2	1.7
1.46×10^{-3}	0.59	10.3	3.4	1.6
3.65×10^{-3}	0.73	16.0	4.7	1.5
7.30×10^{-3}	0.81	22.7	5.8	1.9
18.30×10^{-3}	0.99	25.0	10.5	1.9

The donor contained approximately 20% (by weight) of phenyl acetylene, and its concentration was constant at 10^{-3} M.

Depolarisation ($\bar{P}$) was measured using the equation[3] $\bar{P}=\dfrac{I_{VV}+GI_{VH}}{I_{VV}-GI_{VH}}$

Depolarisation (1) was obtained by exciting the donor and measuring the depolarisation of the acceptor emission: $\lambda_{ex}=285$ nm; $\lambda_{em}=442$ nm. Depolarisation (2) was obtained by exciting the acceptor and measuring the depolarisation of the acceptor emission: $\lambda_{ex}=410$ nm; $\lambda_{em}=472$ nm. Depolarisation (3) was obtained by exciting the donor and measuring the depolarisation of the donor emission: $\lambda_{ex}=285$ nm; $\lambda_{em}=380$ nm.

The results for the polymers have two possible interpretations: either (1) intramolecular energy transfer for these polymers does not occur to any significant extent by the dipole–dipole mechanism and therefore the currently held theory of energy migrating along the polymer chain into traps is incorrect, or (2) the probability of transfer does not decrease as $\cos^2$ (the angle between moments) but at some higher power. The data presented in Table 1 show that intermolecular transfer is occurring for perylene and consequently the $\cos^2$ relationship holds.

We conclude that energy transfer along polymer chains does not take place by the Förster-type non-radiative mechanism. Further experiments are being carried out to attempt to elucidate the mechanism of intramolecular transfer for polymers.

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Large ground deformation on Mount Etna volcano

AN 11-km traverse of benchmarks for precise levelling across the summit of Mount Etna was established and levelled in July 1975. The traverse runs from a reference benchmark near the Piccolo Rifugio (about 2515 m a.s.l.) in the south, across the summit cone (highest station about 3210 m a.s.l.) and down to the rim of the Cratere Ellittico Caldera near Pizza Deneri (about 2780 m a.s.l.) (Fig. 1). The traverse is re-occupied about twice a year to monitor vertical ground movements associated with volcanic activity, the method being similar to that used on Kilauea Volcano, Hawaii[1–3]. A Zeiss Ni2 self-levelling level with micrometer and 3-m invar staff are used and precise levelling techniques adopted where possible. (Previous precise levelling on Etna[4] was restricted to dry tilt stations at three localities.) Closures on ½-km traverses are better than first order accuracy. Re-levelling has so far been carried out in May 1976 and

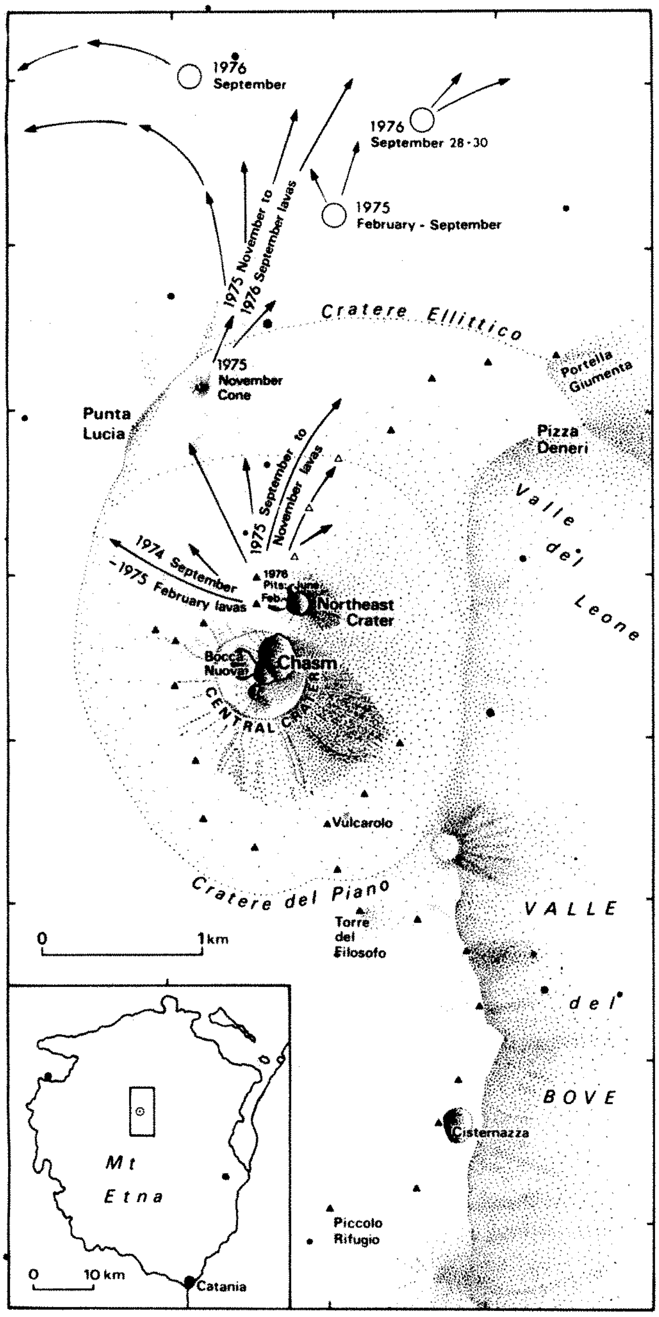

Fig. 1 Map of Mt Etna summit, showing the activity 1974–76, and the location of the levelling traverse. ▲, Principal benchmark; △, principal benchmark destroyed by lavas of September to November 1975; ○, approximate centre of quiet emission of lava; arrows show directions of lava flows.

September 1976, and takes about 6 d for the complete traverse. We report here the results of this repeated precise levelling, which show that an area at least 2 km across subsided >1 m between July 1975 and September 1976. This could indicate high-level removal of magma, or incipient caldera collapse.

After being dormant for 3½ yr following the 1971 eruption, the North-east Crater started activity again on 28 September 1974, with strombolian activity quickly followed by lava eruption. This activity ceased on 23–24 February 1975, when lava began erupting from new boccas 2 km to the north at about 2,500 m a.s.l. (Fig. 1). The rate of eruption was measured up to 1.5 $m^3 s^{-1}$ during this phase of eruption, which lasted until 12 September. During the first occupation of the traverse (30 July–5 August 1975) lava was still coming out intermittently at this site, occasionally ceasing for a few days, when minor strombolian activity and internal collapse occurred at the North-east Crater. This alternation between emission of lava on the north flank and activity at the North-east Crater continued until 12 September, when north flank effusion stopped, and lava accompanied by strombolian activity erupted from the North-east Crater and began new flows down the northern slopes. These buried >1 km of the northern jeep track, and destroyed 14 of our benchmarks. Rates of eruption of 0.9 $m^3 s^{-1}$ were measured. This activity stopped on 28 November 1975, and on 29 November a new fissure opened 450 m ENE of Punta Lucia (altitude ~2900 m a.s.l.), with eruption of lava and violent strombolian activity, which rapidly built up a cone about 40 m high. In early 1976, a collapse pit about 120×70 m formed on the west flank of the North-east Crater, accompanied by considerable emission of ash and the onset of intense sulphur-bearing fumarolic activity.

At the time of the second occupation of the traverse, 20–26 May 1976, activity near Punta Lucia continued with intermittent weak strombolian activity in the cone and emission of lava at a rate of 1–3 $m^3 s^{-1}$. Three weeks after the leveling on 17 June, a new summit vent opened on the north-west flank of the North-east Crater, and strombolian activity ensued, which, assisted by some collapse, produced a crater about 80 m wide. Strombolian activity continued until 20 August, interspersed with a 3-week period of high-pressure gas emission in late July. Quiet effusion of lava near the Punta Lucia vent followed, but by the time the traverse was levelled for the third time (16–21 September 1976) large flows were emerging at a fairly high rate (3–4 $m^3 s^{-1}$) much further down the northern side, >3 km north of the summit at an altitude of about 2,300–2,400 m.

In May 1976, unusually late snowdrifts prevented re-levelling of the northern half of the traverse, apart from the final 500 m. Vertical movements in this and the southern section proved small, but two isolated benchmarks 40 m apart protruding above the snow 300 m west of the North-east Crater were re-levelled, and gave 3.5 cm of relative downward movement towards the north-east.

In September 1976, new benchmarks were set up on the September–November 1975 lava, so that the whole traverse could be re-levelled. Changes in altitude since May 1976 were again small in the southern section, −1.3 cm being the largest movement; but in the northern section, inacces-

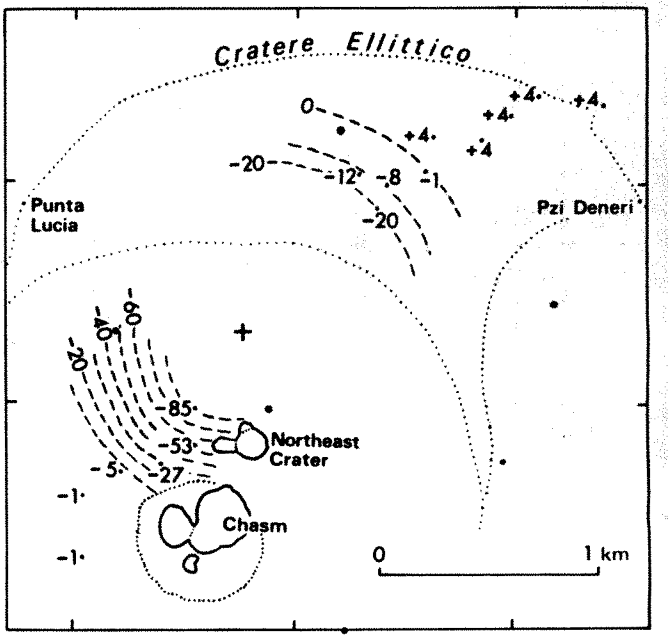

Fig. 2 Map showing relative changes in altitude (cm) of benchmarks between July 1975 and September 1976. – – – –, Inferred 10-cm contours of altitude change. +, The presumed centre of collapse.

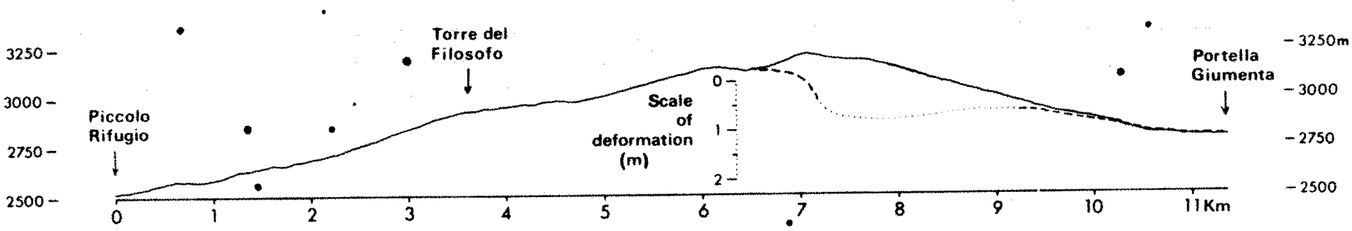

Fig. 3 ——, section of the levelling traverse (vertical exaggeration ×2); – – –, measured ground deformation between July 1975 and September 1976 with ×500 exaggeration, relative to the profile.

sible in May, the changes since July 1975 are very large. They indicate collapse of an area >2 km wide, from just north-east of the Central Crater in the south, to just south of the Cratere Ellittico in the north (Fig. 2). On the southern side of this area of deflation, the ground deformation increases northwards from −0.9 to −84.6 cm in only 700 m, and assuming a fairly smooth profile of deformation (Fig. 3) the amount of subsidence at the centre of collapse is likely to be >1 m. The maximum amount of collapse cannot be determined exactly since the centre of collapse lies in the unmonitored area where our benchmarks were destroyed. To the north of the unmonitored area, the amount of ground collapse decreases northwards from −19.5 to +4 cm, at which slightly inflated value it remains almost steady over the most northerly 800 m of the traverse.

About 1 m of vertical deformation in 1 yr is large, considering that no major eruption took place during this time. The most unusual feature of this subsidence is the small diameter (just over 2 km) of the area affected. This is unlike inflation and deflation on Hawaii, where the whole volcano is affected[1–3]. The actual tilt of the ground implied by these measurements is at least 1,200 μrad on the south side of the area of collapse.

The large amount of vertical deformation compared with the small horizontal extent of the area affected suggests that the cause of the deformation is at a shallow depth, and could result from several causes: one is that as the area of subsidence contains the North-east Crater flows of 1966–71 and 1974–75, we are observing cooling contraction and compaction of these flows. We think this unlikely especially as the 1971 flows on the south side of the summit cone show no such movement. Also, precise level tilt stations on older flows outside the area indicate deflation. Another more likely explanation is that the collapse could be directly related to the summit activity of 1975 September–November and 1976 June–August. One might expect the summit to be inflated in late July 1975, 6 weeks before summit activity, and deflated in September 1976, 1 month after summit activity. The levelling of the two isolated summit benchmarks in May 1976 shows that most of the collapse had already taken place at that time. This is likely to be the case since the summit activity in August was relatively minor. Finally, we could be witnessing incipient caldera formation, and that collapse could continue to take place in the area. Caldera formation has occurred on Etna at intervals of several hundred years, and the recent occurrence of other types of collapse at the summit, such as the collapse of the Bocca Nuova since 1970 and the collapse of the two new pits on the North-east Crater in early 1976 and June 1976 could support this view. The total void created by these three events is about 5×10^6 m³, added to an estimated 1×10^6 m³ created by the ground deformation. The volume of lava erupted during this time is at least an order of magnitude larger.

Whichever of the above possibilities proves to be correct, the interest of these results and the importance of continuing these observations is self-evident. They contribute to a greater understanding of the internal structure and workings of the volcano, which will hopefully lead to an increasing ability to predict activity, and point to areas of interest for other studies on the mountain.

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Evidence for a low velocity layer at the base of the oceanic crust

As the quantity of seismic refraction data in the oceans increases it is becoming apparent[1–3] that there is a systematic increase in crustal thickness with age, at least out to about 60 Myr (refs 1, 2). To explain this phenomenon more precisely the detailed structure of the lower crust is required. We present seismic refraction data from the Northern Cocos plate which shows that thickening occurs by the formation of a low velocity zone (LVZ) at the base of the crust with a velocity

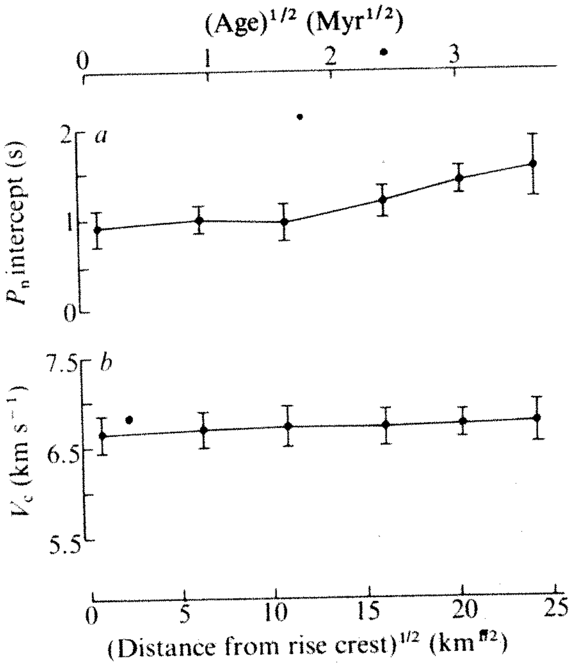


Fig. 1 *a*, Average mantle intercept times (P_n) and *b*, average crustal velocities V_c (ref. 2) against (age)$^{\frac{1}{2}}$ in (Myr)$^{\frac{1}{2}}$. The spreading rate is about 4.5 cm yr^{-1} and the error bars represent 1 s.d. The intercept times have had the travel time through the water column subtracted.

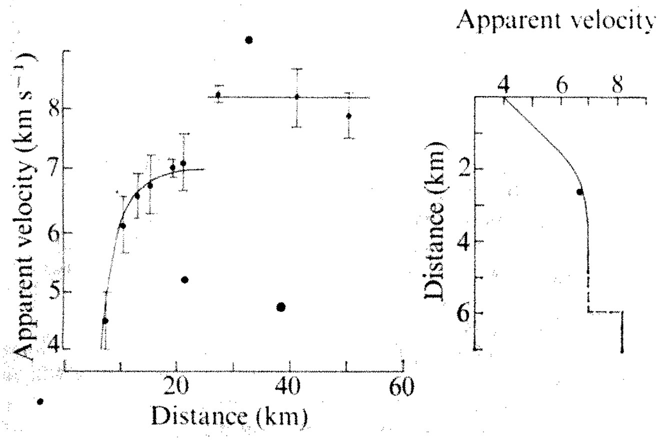


Fig. 2 Apparent velocities of first arrivals (with 1 s.d. error bars) measured across the array of receiving buoys (line 67).

< 6.8 km s^{-1}. One possible explanation of this in terms of these relatively low seismic velocities for the lower crust[4], is that upper mantle material becomes serpentinised by a hydration reaction as it cools. If so, this implies that the Moho boundary, except at very young ages, is a phase change (hydration) boundary. This supports the ideas of Hess[5].

Our data were taken over the Northern Cocos plate in an area bounded on the north and south by the Orozco and Clipperton fracture zones and on the east and west by the Middle American Trench and the East-Pacific rise (EPR). The spreading rate in this area is about 4.5 cm yr^{-1} (ref. 6). We have some 40 refraction lines each about 80 km long covering the area on an orthogonal grid. Most data were obtained from free-floating buoys and some from ocean bottom seismometers, using standard seismic refraction techniques. The upper mantle velocities obtained from these data, which show strong anisotropy, are discussed by Snydsman *et al.*[7].

Using only the first arrivals, the mantle intercept times and crustal velocities were measured and then averaged in age intervals after correcting for the water column travel time.

Fig. 4 Graph for line 8 showing trade-off between LVZ parameters and thickness of the overlying layer, found using the PmP critical point.

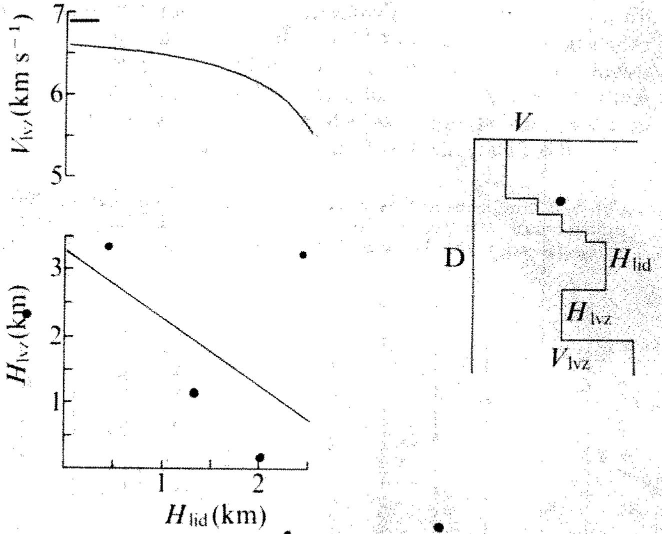
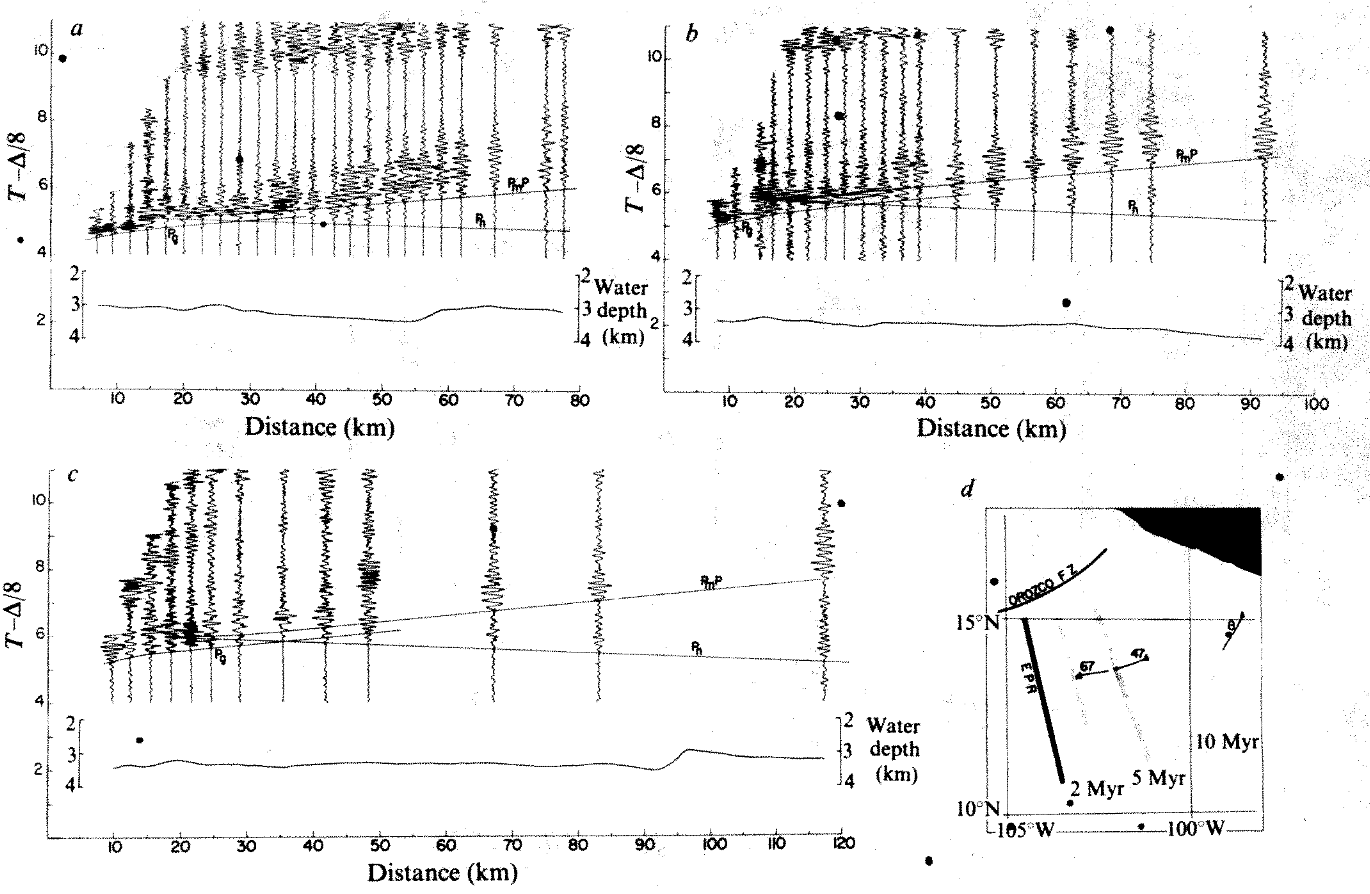


Fig. 3 Record sections from seismic refraction data taken at: *a*, 165; *b*, 275, and *c*, 600 km from the rise crest (lines 67, 47 and 8). The data are obtained by firing a sequence of explosive shots along a line from an array of telemetering buoys. The seismograms are then plotted on a reduced time scale, defined as $T_{\text{reduced}} = T_{\text{observed}} - \text{distance}/8.0$. On this scale horizontal lines correspond to 8.0 km s^{-1}. The travel time curves on each record section are interpretations based on correlating arrivals from one record to another. *d*, Base chart of area studied.



These averages were then plotted against $(\text{age})^{1/2}$ (Fig. 1). The $(\text{age})^{1/2}$ abscissa was chosen since the most rapid changes due to cooling can be expected in the early history of the crust[8]. From Fig. 1*a* it is clear that the averaged intercept time increases with age by about 0.5 s in 12 Myr. Of this increase only about 0.1 s could be associated with an increasing upper mantle velocity. We therefore conclude that the crust is thickening with age.

In obtaining the upper crustal structure we have used both the travel times and the apparent velocities measured across the 3.0-km long array of five receiving buoys. An example of the apparent velocities we obtained is provided by the data from line 67 (Fig. 2). This line was notable for the small drift of the buoys (< 1 km in 4 h) and the smooth topography. The travel times for this line are shown by the unfiltered record section in Fig. 3. In deriving a model for the lines, it was found that the simplest model which fits both the travel time and apparent velocity data within the observational error was a roughly linear velocity increase in the upper crust followed by a decrease in the gradient with depth. Theoretical apparent velocities from such a model are shown in Fig. 2 superimposed on the data. Although there is some smoothing of the apparent velocities introduced by the finite length of the array the existence of a discrete velocity jump associated with the layer 2–layer 3 boundary clearly is not supported, since this would appear as a discontinuity in the apparent velocity curve near 15 km. This conclusion is supported by all the lines for which we have good array velocity data and also by the results of Orcutt *et al.*[10]. The layered velocity model of the crust, usually assumed, seems to be a simplification and approximation but not a realistic physical model in this area.

To examine the crustal thickening with age, record sections were constructed of selected lines and velocity models which fit both first arrivals and later arrivals were constructed using ray tracing methods, synthetic seismograms and critical point locations. Three such lines (oriented perpendicular to the

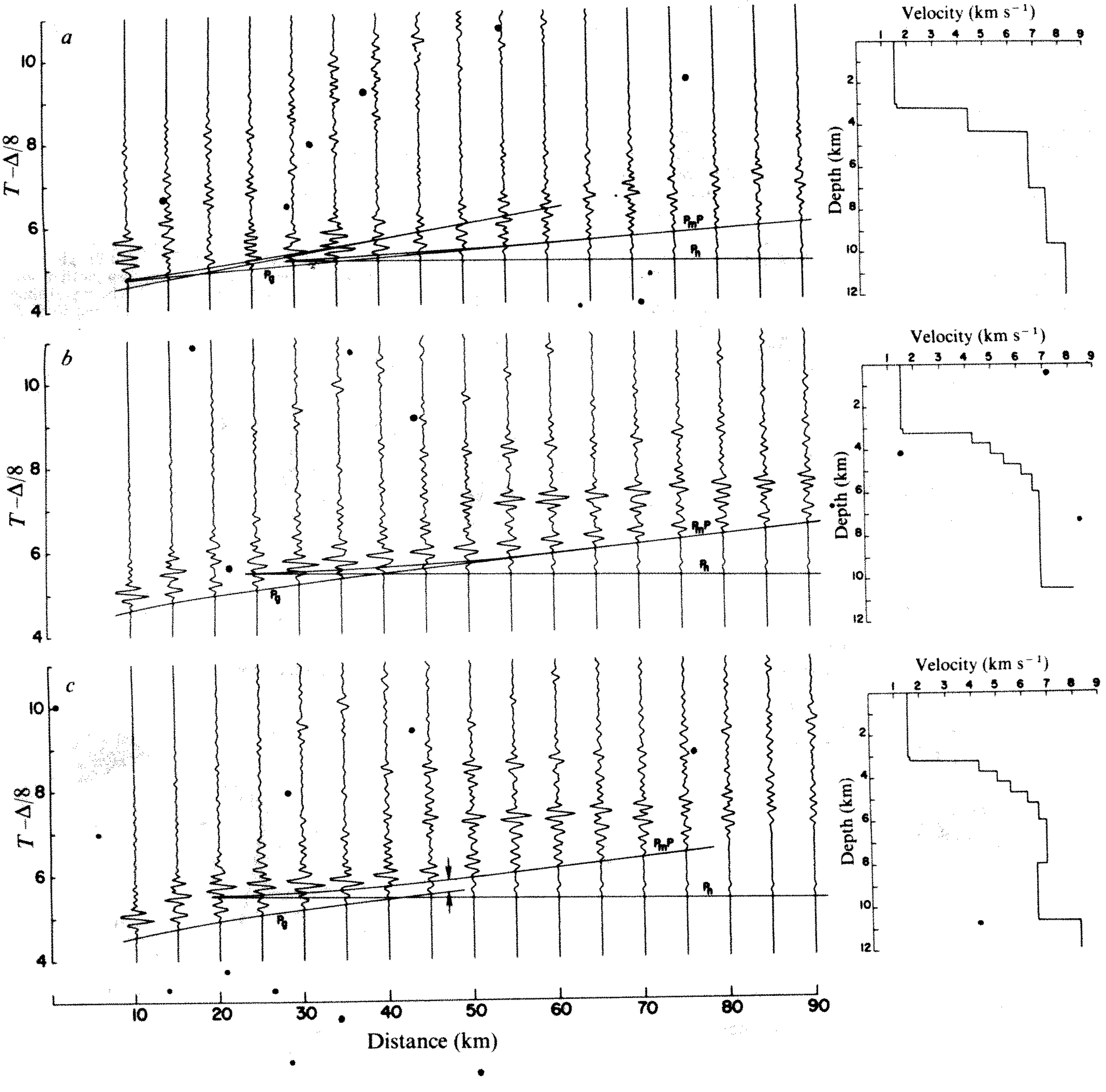


Fig. 5 A comparison of synthetic seismograms computed for three types of models. Model *a* has a basal high-speed layer. Model *b* has an upper crustal structure derived from our travel time and apparent velocity data and a constant velocity lower crust. Model *c* is similar to Model *b* except for the basal LVZ. Note the P_mP–P_g offset and the decrease in the P_mP–P_n critical point distance produced by the LVZ.

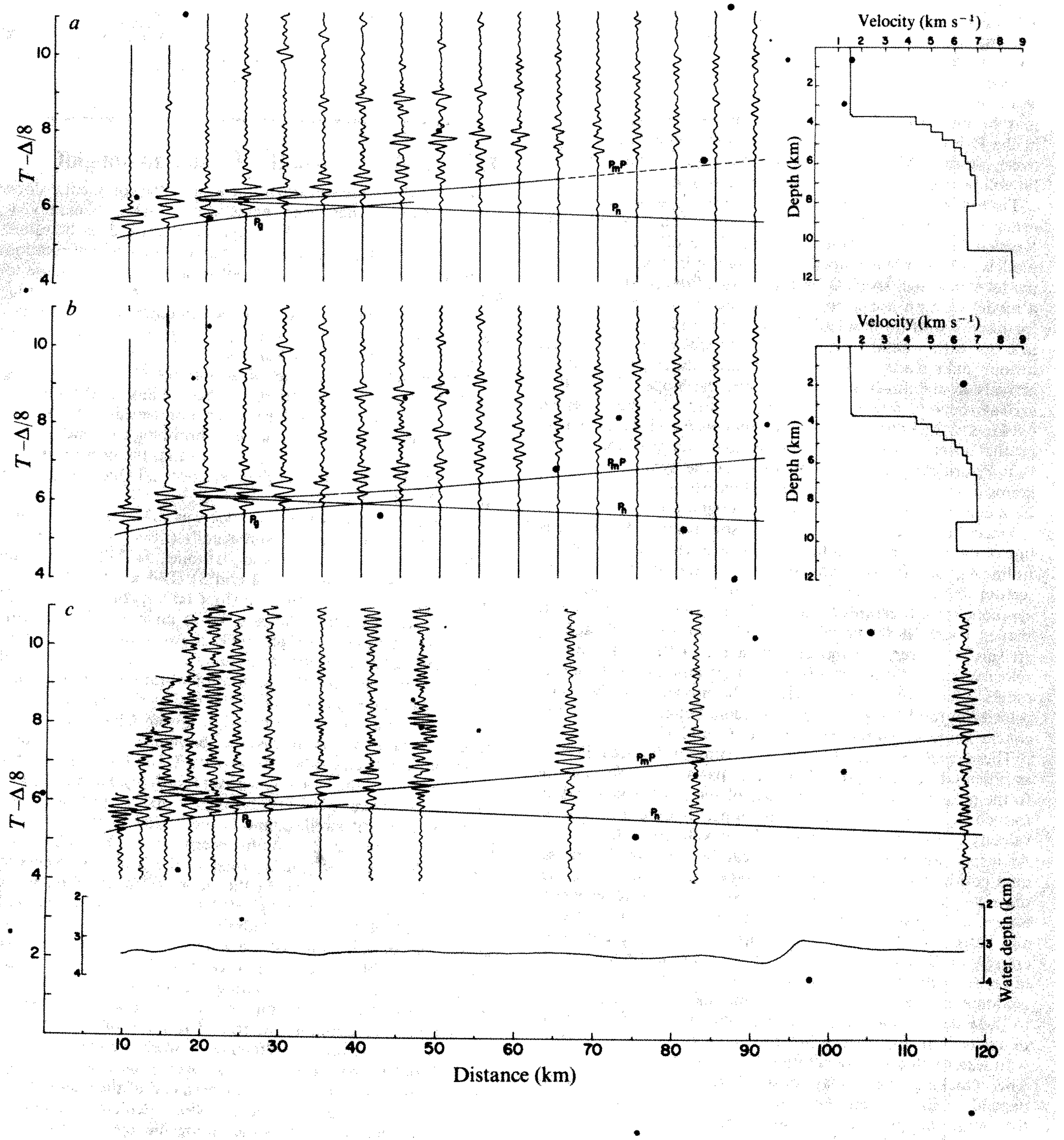


Fig. 6 A comparison of synthetic seismograms computed from the models shown (*a*, *b*) with line 8 (*c*). Model *b* with the thin LVZ fits the P_mP arrivals at large ranges better than *a* and is, therefore, the preferred model.

observed magnetic anomalies) are shown in Fig. 3, together with an interpreted travel time curve. Critical to our interpretation are the large amplitude late arrivals at distances greater than 50 km. These arrivals are wide-angle reflections (P_mP) off Moho[9] and at the largest ranges the apparent velocity, or inverse slope, of these arrivals must be asymptotic to the velocity at the base of the crust or, in the case of a LVZ at the base of the crust, they will be asymptotic to the velocity of the lid overlying the LVZ. One test for the existence of a LVZ is whether these arrivals are tangential to the crustal refracted arrivals or whether they are offset. For line 8 at 600 km from the rise axis there is a clear offset of about 0.35 s. The other two lines also have a well-marked offset of about 0.2 s. This effect is most easily seen by comparing these lines with lines close to the axis (not shown here) which show no offset. The wide-angle reflections also give the velocity of the lid, and in this case, we found velocities of 6.9, 6.8 and 6.9 km s^{-1} respectively.

A second independent test for the existence of a LVZ is the distance and time at which the P_mP critical point occurs on the travel time curves. This, coupled with a knowledge of the upper crustal structure and P_n velocity, can be used to determine if a LVZ exists at the base of the crust. For the lines shown in Fig. 3 the P_mP critical point is fairly clear and we can determine the maximum range at which the critical point may occur. Using the measured critical points, the derived upper crustal structure, and the asymptotic velocity of P_mP, we can determine if a LVZ is required at the base of the crust.

Using this method, we find that our three lines do require a LVZ at the base of the crust. There is, however, a trade-off between the thickness and velocity of the LVZ and the thickness of the lid, as shown in Fig. 4 for line 8. A further constraint may be placed on the LVZ by the distance to which energy in the P_mP path is propagated since the thickness of the layer overlying the LVZ strongly controls this feature (that is, a thick lid will propagate energy further than a thin lid).

To test our model, further, we synthesised seismograms, using the method of Fuchs and Muller[11] as modified by Kennett[12] for marine situations, for three different types of models. These models labelled *a*, *b*, and *c* in Fig. 5 include a model with a high-speed layer at the base of the crust (model *a*), a model with an upper crustal structure similar to that found for line 67 with no basal LVZ (model *b*), and a model similar to *b* but with a basal LVZ (model *c*). Model *a* clearly does not fit our data since it does not produce the high amplitude arrivals in the 15–20-km range; also, the slope of the P_mP arrivals beyond 50 km is 7.5 km s^{-1} and not the observed 6.8 km s^{-1}. The difference between models *b* and *c* is primarily in the offset between P_mP and P_g and the position of the P_mP–P_n critical point. A comparison of the synthetic seismograms in Fig. 5 and the data in Fig. 3 clearly supports the basal LVZ concept and the criteria for its recognition.

Since the models in Fig. 5 are not specifically related to any line of data, we next tried to compute synthetic seismograms to fit line 8 as accurately as possible. Fig. 6 shows the line 8 record section bandpass filtered from 0 to 8 Hz along with two synthetic record sections computed from models with differently shaped LVZs. Both models give a close fit to the P_g and P_n arrivals; however, the model with a thin LVZ and a higher velocity contrast fits the observed P_mP amplitudes better at ranges greater than 50 km. Hence the model with the lower velocity in the LVZ seems to fit the data better for line 8; this conclusion was also reached for lines 67 and 47.

These results suggest that the thickening of the oceanic crust as observed here and possibly in other parts of the world is due to the growth of a LVZ at the base of the crust which may be formed by a transformation of upper mantle material of velocity > 8.0 km s^{-1} into material with velocity $\lessdot$ 6.8 km s^{-1}. An inspection of the metamorphic reactions that might operate on a peridotite-type rock in a cooling environment to produce these low velocities suggests only one candidate, a serpentinisation or hydration of the peridotite. The data of Christensen and Salisbury[4] show that if the upper mantle were composed entirely of olivine then a 50% hydration would be required to lower the velocity from 8.43 to 6.5 km s^{-1}. If this serpentinisation hypothesis is correct, then a method of getting the water to these depths may be provided by the cracking front theory of Lister[13]. In this theory hot rock cooled rapidly by contact with water will crack, facilitating further cooling and cracking. This cracking front may proceed to considerable depths depending in part on the strength of the rock. Lister[13], in discussing this problem, had suggested such a mechanism for the formation of the Moho boundary.

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J. Tuzo Wilson Knolls: Canadian hotspot

A YOUNG submarine volcano at the base of the continental slope west of Canada has been named after the Canadian geophysicist J. Tuzo Wilson. The volcano is capped by hawaiite, and has an angular separation from a proposed pole of rotation of the Pacific plate and the hotspot frame of reference close to separations for the Kodiak–Bowie seamount chain, suggesting that a hotspot which generated the seamount chain now lies beneath the young volcano. Radiogenic ages of some seamounts of the chain support this hypothesis.

The generation of a line of seamounts (Fig. 1), trending south-east from Kodiak to Bowie Seamounts in the Gulf of Alaska, has been ascribed to a hotspot beneath the Pacific Plate[1,2]. Bathymetric, geometric, chronological and petrological evidence presented here suggests that the hotspot is now located at the foot of the continental slope, off Queen Charlotte Sound, British Columbia.

In 1973, fresh glassy volcanic rock was raised in two dredge-hauls made on J. Tuzo Wilson Knolls (JTW) at latitude 51° 23′N, longitude 131°02′W and latitude 51°25′N, longitude 131°01′W, respectively (Figs 2 and 3). The knolls which rise from the base of the continental slope 60 km south of Cape St James, Queen Charlotte Islands, lie in an area of high sedimentation rate. The dredged rock shows little sign of manganese encrustation or alteration, and most likely was erupted recently, probably since the diminution of rate of sedimentation following the rise in sea level at the end of the last glaciation. The knolls lie at the southern end of Queen Charlotte fault, a transform fault which forms the boundary between North American and Pacific lithospheric plates[3–5] and lies north of the triple junction of the North American (NOAM), Pacific (PCFC) and Juan de Fuca (JDFA) lithospheric plates[5]. Oshawa Rise, a broad basement swell, connects the region of the knolls to Bowie Seamount, at the south-eastern end of Kodiak–Bowie Chain[1,4]. Minster *et al.*[6], in a global synthesis of movements of lithospheric plates relative to the hotspot frame of reference (HSPT), computed that PCFC–HSPT rotation during the past 10 Myr has been 0.83° Myr^{-1} about a pole at 67.3°N, 120.6°W. Colatitudes of seamounts of the Kodiak–Bowie Chain relative to this pole (Table 1) are in the range 36.9°–39.4°. The colatitude of JTW is 37.1°. After calculating the longitudinal difference between JTW and a seamount of the chain about the PCFC–HSPT pole, and assuming the rate of rotation of 0.83° Myr^{-1}, one may calculate the age difference between the seamount and JTW (Table 1, rows 4 and 5). Radiogenic ages of Giacomini and Kodiak seamounts, at the northern end of the chain (Table 1, rows 6 and 7) compare closely with calculated seamount–JTW age differences, as does the radiogenic age of basalt from nearby DSDP hole 178. Radiogenic ages of Bowie, Hodgkins and Dickens seamounts, near the southern end of the chain, are 5–6 Myr younger than the calculated age differences. Assuming that volcanism, once initiated at a seamount, has a duration of several Myr, such age discrepancies are not incompatible with the hypothesis that the active end of Kodiak–Bowie Seamount Chain lies at or near JTW, and that the rotation PCFC–HSPT has been constant for the past 22 Myr. Alternatively, the discrepancies may indicate variation in PCFC–HSPT motion through time.

Other possible locations for the active end of the Kodiak–Bowie chain on the Pacific plate are Dellwood Knolls and the south-eastern end of Dellwood Seamount Range (Figs 1 and 2), relevant colatitudes of which are 37.4° and 37.8°, respectively. The south-eastern end of Dellwood Seamount Range lies at the

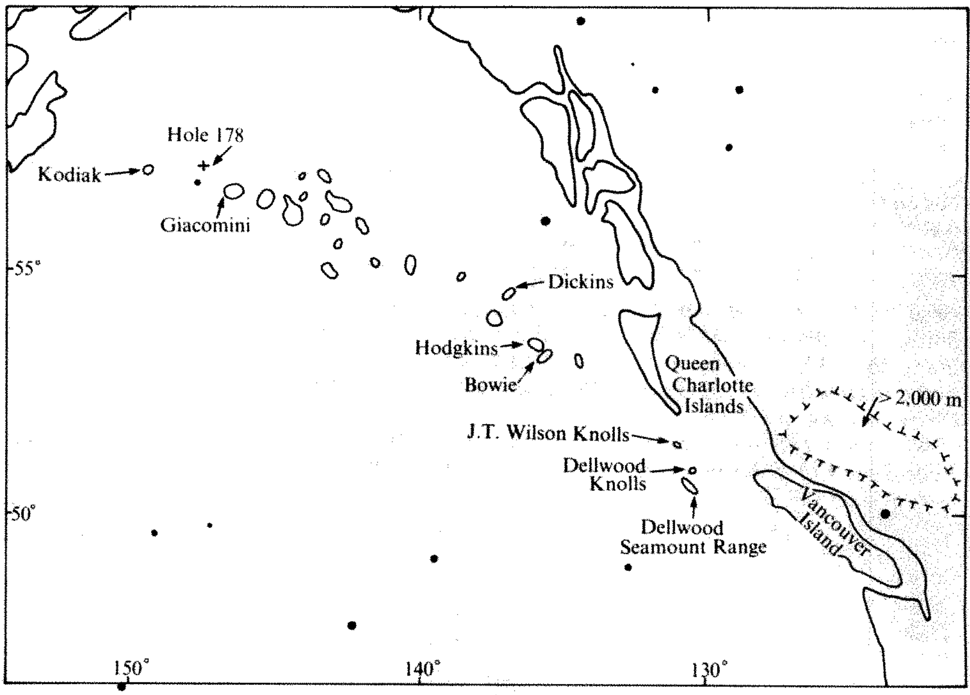

Fig. 1 Location of Kodiak-Bowie Seamount Chain in Gulf of Alaska. Ticked dashes enclose area of Coastal Mountains of southwestern British Columbia characterised by elevations > 2000 m.

northern end of Explorer Ridge. Dellwood Knolls lies to the east, on the eastern side of Revere–Dellwood fracture zone[5,7] (Fig. 2). Basalt without significant coating of manganese–iron oxides has been recovered at both of these sites. Analyses of major elements and norms of volcanic rock from JTW, Dellwood Knolls and Dellwood Seamount Range (Table 2) reveal that the latter two features are composed of low potassium tholeiite characteristic of spreading ridges, whereas JTW is composed of much more alkaline hawaiites, characteristic of the terminal phases of seamount volcanism. Thus petrology indicates that JTW is the most likely of the three features to be generated by a hotspot.

If initiation of volcanism at JTW occurred several Myr ago, the generating hotspot would now lie east of the plate margin under the North American plate. If this is true, geometry dictates that the hotspot lies on the same small circle about the PCFC–HSPT pole as the Kodiak–Bowie chain. A broad positive topographic anomaly and alkaline volcanicity should characterise such a hotspot[9]. In the coastal mountains of British Columbia, a topographic high does occur, centred at 51°N, 125°W (Fig. 1), but the colatitude (PCFC–HSPT) of this area is only 35°, and no young alkaline volcanicity is known from it.

Thus the tentative conclusion is that the Kodiak–Bowie Hotspot is at present located near J. T. Wilson Knolls.

The decision to name this topographic feature after the Canadian geophysicist J. Tuzo Wilson stems principally from its connection with an underlying hotspot. Professor Wilson was the originator of the hotspot hypothesis, and it is thus fitting that a hotspot be named after him, particularly one

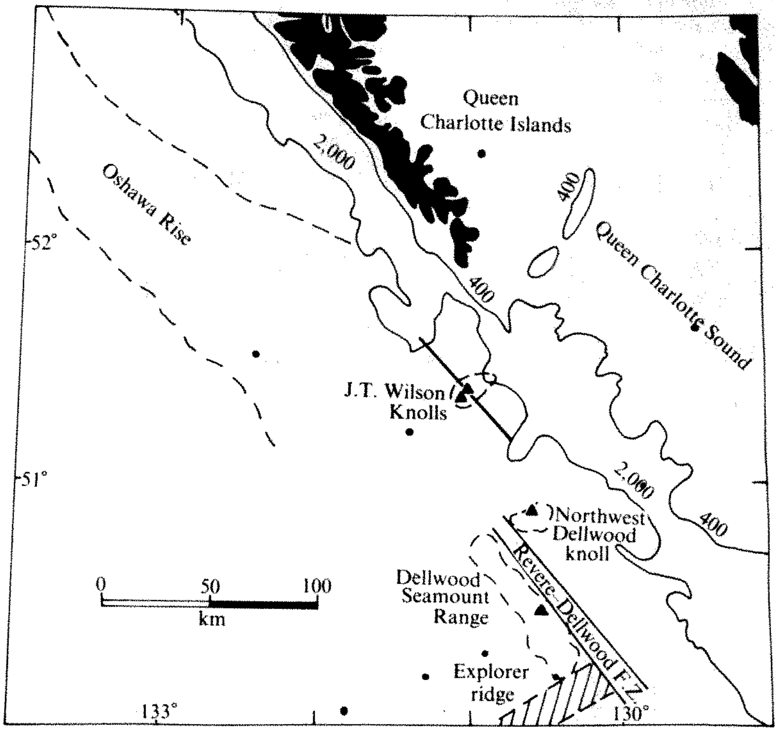

Fig. 2 Relation of J. Tuzo Wilson Knoll to neighbouring features. Queen Charlotte Fault follows continental margin off Queen Charlotte Islands. Heavy straight line indicates locations of continuous seismic reflection profile (Fig. 2). Triangles indicate sites of dredged rock for which analyses are listed in Table 2. Contours in m.

Table 1. Position and age data for Seamounts studied

	J. T. Wilson	Bowie	Hodgkins	Dickens	Giacomini	DSDP 178	Kodiak
Co-latitude about PCFC–mantle Pole (deg)	37.1	37.4	37.4	36.9	38.8	38.6	39.4
Angular distance from JTW about PCFC–mantle Pole(deg)	0	5.5	6.1	7.8	16.6	17.6	18.9
Calculated age (Myr)	0	6.7	7.4	9.4	20.0	21.2	22.8
K/Ar age (Myr)	—	0.1±0.1	2.65±0.2	3.7±0.2	19.9±1.0	22–23	22.6±1.1
Fission-track age (Myr)	—	—	—		19.3±3.8		25.3±4.3
Age of magnetic anomaly (Myr)	<10	18	19	20	46	47	50
Age–difference (Myr) magnetic-calculated	—	11	12	11	26	26	27

close to Canada. He introduced the concept of transform faults and, with Frederick Vine, first delineated plate boundaries in the north-eastern Pacific. Thus it is fitting that his hotspot lie in the north-eastern Pacific, near the southern end of the Queen Charlotte transform fault.

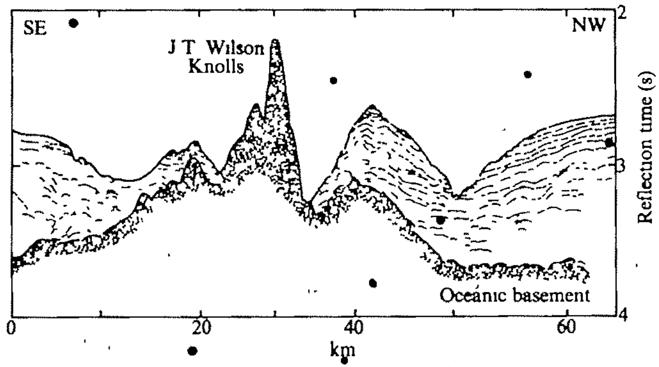

Fig. 3 Continuous seismic reflection profile across J. Tuzo Wilson Knoll. Sedimentary strata cut by canyons of the continental slope cover flanks of volcanic edifice rising from oceanic basement. Vertical exaggeration is 21:1.

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Table 2. Analyses of major elements and norms of volcanic rocks

	Hawaiite J. T. Wilson Knolls	Hawaiite J. T. Wilson Knolls	Qz-tholeiite Dellwood Smt Ra.	Ol-tholeiite Dellwood Knolls
SiO_2	49.47	51.47	50.38	47.48
TiO_2	2.24	1.51	1.48	1.16
Al_2O_3	17.25	17.83	14.71	15.87
Fe_2O_3	1.67	1.23	1.80	1.29
FeO	6.75	5.17	7.38	7.69
MnO	0.14	0.13	0.18	0.16
MgO	4.52	5.06	7.12	10.71
CaO	7.95	8.16	12.53	11.26
Na_2O	4.94	4.74	2.51	2.21
K_2O	2.47	2.26	0.26	0.18
H_2O^+	1.36	1.56	1.95	0.64
H_2O^-	0.45	0.33	0.03	0.09
P_2O_5	0.92	0.77	0.13	0.09
	100.13	100.22	100.46	98.83
Q	—	—	0.33	—
Or	14.60	13.35	1.54	1.06
Ab	27.53	32.12	21.24	18.70
An	17.60	20 70	28.10	32.85
Ne	7.73	4.33	—	—
Di	12.93	11.94	26.98	18.08
Hy	—	—	14.58	6.82
Ol	9.13	9.46	—	16.30
Mt	2.42	1.78	2.61	1.87
Il	4.25	2.87	2.81	2.20
Ap	2.14	1.79	0.30	0.21

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Cerium in chert as an indication of marine environment of its formation

THERE are few if any investigations of deep-sea cherts or siliceous sediments from the geochemical point of view, although several geological and mineralogical studies have been published[1–6]. We present here data on rare-earth elements (REE) in deep-sea cherts, and compare them with relevant data on seawaters as well as cherts exposed on land.

Behaviour of cerium differing from that of other REE in marine environments is already known[7–10] with particular reference to manganese geochemistry, but a similar distinction in predominantly siliceous sedimentary rocks has not been reported, to our knowledge.

REE in samples were determined with stable isotope dilution technique with relative uncertainties of <1.5%. Our investigations were performed on four deep-sea cherty rocks recovered during the Deep Sea Drilling Project, two microfossil separates, and three terrestrially exposed cherts, including an Archaean one from Gunflint, Canada. The microfossils were separated from radiolarian ooze by the sedimentation technique. The microfossils under consideration are siliceous and substantially free from calcareous ones[11]. Excepting 4-29C-1 (chert from the Caribbean Sea), the other deep-sea samples are from the central Pacific.

The results of REE determination are given in Table 1. Figure 1 shows the chondrite-normalised REE patterns for deep-sea samples; the REE abundances in the Leedey chondrite[12] are employed for the Masuda–Coryell plotting (plotting the logarithm of chondrite-normalised value against the order of atomic number of REE). It should be noted that all of the DSDP samples investigated show large negative Ce anomalies. The 'Ce anomaly' here is defined as the ratio of observed Ce abundance to that which would fall on the La–Nd join in the Masuda–Coryell plot. The Ce anomaly ranges approximately from 0.2 to 0.3, with the exception of the Caribbean chert (0.467).

The absolute concentration levels of REE in the four highly siliceous sediments show a large variation decreasing in the order 7-66-5, 16-163-14, 4-29C-1 and 17-167-85. Sample 7-66-5 is cristobalitic porcellanite, the next two samples are cristobalite cherts, and the last one, 17-167-85, is a quartz chert. Thus, the

Table 1 REE concentrations (p.p.m.) in cherts and siliceous microfossils, with sample descriptions

	DSDP 7-66-5	DSDP 4-29C-1	DSDP 16-163-14	DSDP 17-167-85	DSDP 7-66-3 microfossil	DSDP 16-162-7 microfossil	750824-14	750824-15	Gunflint
La	36.7	12.35	14.11	3.97	5.67	0.311	4.77	5.15	0.0785
Ce	24.4	12.16	6.62	2.18	2.98	0.1519	10.04	14.94	0.1946
Nd	35.7	12.75	15.62	2.92	6.96	0.355	4.56	5.45	0.0738
Sm	8.03	2.72	3.50	0.531	1.569	0.0797	0.920	1.250	0.01536
Eu	2.08	0.706	0.906	0.1295	0.395	0.01994	0.202	0.271	0.00453
Gd	9.03	3.08	3.75	0.645	1.864	0.0666	0.866	1.115	0.01790
Dy	9.21	2.87	3.81	0.598	1.835	0.0802	0.727	1.024	0.0222
Er	5.62	1.674	2.27	0.388	1.157	0.0458	0.451	0.544	0.01681
Yb	—	1.440	2.03	0.355	1.059	—	0.445	0.492	0.01632
Lu	0.842	0.219	0.316	0.0551	0.1893	0.00669	0.0672	0.0731	0.00236
Ce anomaly*	0.321	0.467	0.217	0.290	0.235	0.224	1.02	1.37	1.21
Latitude Longitude Water depth(m)	2°23′N 166° 7′W 5293	14°47′N 69°19′W 4247	11°15′N 150°18′W 5320	7°4′N 176°50′W 3176	2°23′N 166° 7′W 5293	14°52′N 140° 3′W 4854	Kamiasô Gifu, Japan		Sibley Ontario, Canada
Age at top	Oligocene–Upper Cretaceous	Middle Eocene	Middle Eocene–Upper Cretaceous	Lower Cretaceous	Oligocene	Middle Eocene	Permian–Jurassic		2.0–2.4 $\times 10^9$ yr
Siliceous microfossils	rare	common	none	none	abundant	abundant	abundant	abundant	none
Lithology	cristobolitic porcelanite	chert	chert	chert	radiolarian ooze	radiolarian ooze	chert	chert	chert
SiO_2 mineral	cristobalite (opal-CT†)	cristobalite (opal-CT†)	cristobalite (opal-CT†)	quartz			quartz	quartz	quartz

*See text.

†After the opal classification by Jones and Segnit[19].

absolute REE abundance levels in these cherty rocks seem to depend on the degree of diagenesis. With the present limited data, it is hard to discriminate on relative REE patterns between cherty rocks and microfossil separates. In general, La, Nd and Sm fall on a straight line, while Sm, Gd and Dy fall on another straight line; there is a small break at Sm.

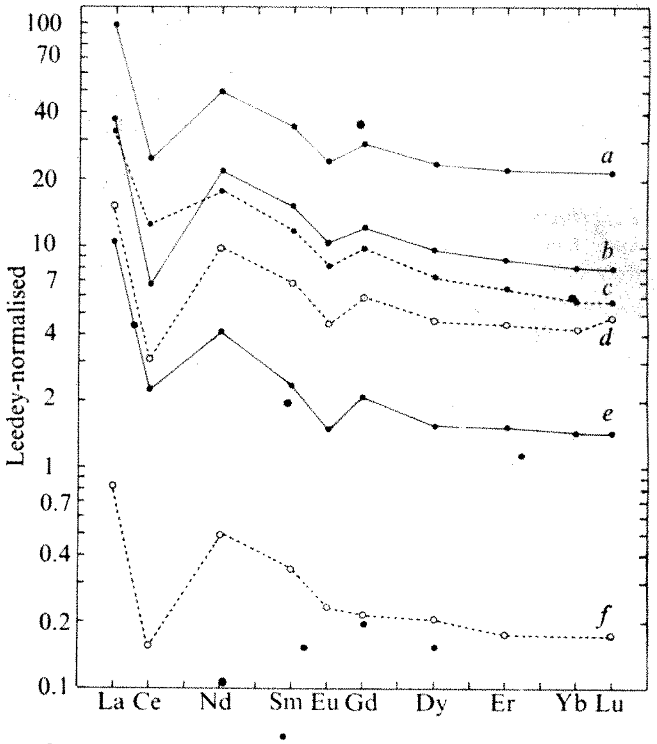
Figure 2 shows the chondrite-normalised REE patterns of four terrestrially exposed cherts; one is a Transvaal chert analysed by Wildeman and Haskin[13] for REE. We have studied two cherts from Kamiasô, Gifu, central Japan, (Triassic–Jurassic) and a Gunflint chert (2.0–2.2, or 2.43×10^9 yr) (ref. 14), Canada. REE contents in the Gunflint chert are similar to but somewhat lower than in the Transvaal sample (about 2.0×10^9 yr) (ref. 13). A remarkable observation from Fig. 2 is that these cherts exposed on land either show a small positive Ce anomaly or seem to have no Ce anomaly at all, in contrast to deep-sea cherts. Kolodny and Epstein[15] have revealed a clear distinction in $\delta^{18}O$ between terrestrially exposed and deep-sea cherts. They conclude that the lower values of $\delta^{18}O$ of terrestrial cherts compared with DSDP cherts are attributable to the shallower water environment in which the terrestrial cherts were formed. This conclusion is consistent with that derived from the present study.

Goldberg *et al.*[7] found that Ce in a seawater sample was depleted by a factor of 0.23 relative to the adjacent rare-earth elements. The corresponding Ce anomaly for eight Atlantic Ocean samples from two stations (45°N, 16°W and 43°N, 28°W) is about 0.32 as a geometrical mean, on the basis of data presented by Høgdahl[16]. He also reported, however, that Ce is present in normal concentrations in seawaters of the Puerto Rico Trench, the West Deep Cariaco Trench and Barents Sea[16]. It should be noted that these areas are all relatively close to continents and/or the dimensions of the seas in question are small compared with oceans. The water of the Black Sea[17] also does not show a substantial Ce anomaly.

It is evident that the presence or absence of Ce anomaly and its extent and direction of deviation can be a good indicator of aqueous (perhaps marine) environment of formation for cherty rocks. A Caribbean chert indicates a smaller depletion of Ce compared with the Pacific ones. This could be due to the Caribbean Sea being encircled by a chain of islands. The REE patterns of terrestrial cherts can be interpreted as suggesting that they were formed in coastal areas, marginal seas or land-enclosed seas.

Our results suggest that, in shallow-sea areas, Ce perhaps mostly involved in suspended solid particles behaved apparently in the same fashion as other REE, while in deep-sea areas Ce was subjected to selective removal relative to other REE. Difference in residence time of lithogenous suspended particles is one of the major factors responsible for this areal difference. Comparatively long residence time of suspended particles would promote disintegration into finer particles and their further alteration, which would be in turn favourable with chemical

Fig. 1 Chondrite-normalised REE patterns of deep-sea cherts and siliceous microfossils (MF) separated from DSDP radiolarian ooze. *a*, sample 7-66-5; *b*, 16-163-14; *c*, 4-29C-1; *d*, 7-66-3 MF; *e*, 17-167-85; *f*, 16-162-7 MF.



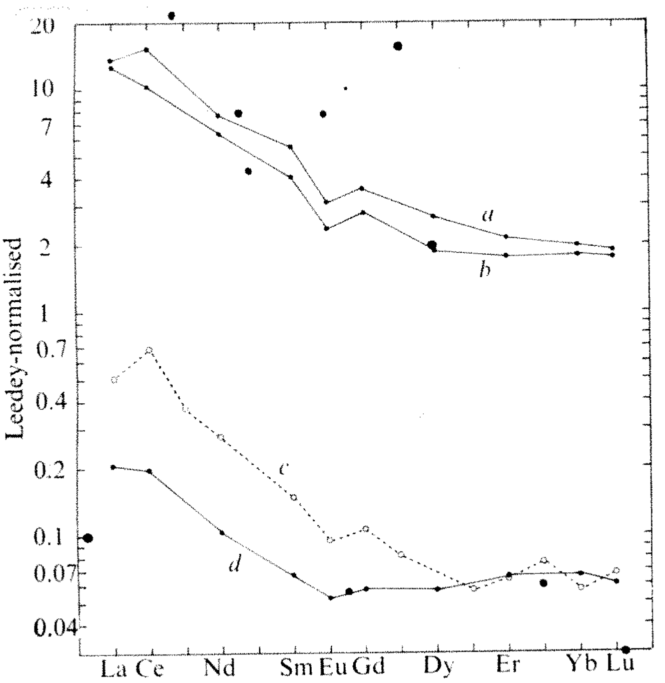


Fig. 2 Chondrite-normalised REE patterns of cherts exposed on land; 750824 samples are from central Japan. *a*, 750824-15; *b*, 750824-14, *c*, Transvaal (Wildeman and Haskin); *d*, Gunflint.

reactions, including oxidation of Ce into the tetravalent form, and separation of Ce from other common REE; without particle disintegration, the size of particles in deep-sea areas can be finer as a result of sorting. This does not necessarily mean that the oxidation into Ce^{4+} starts in deep-sea areas. Høgdahl[18] found no Ce depletion in unfiltered water samples from the River Gironde and its mouth to the sea, while a slight Ce depletion was detected for filtered waters.

Almost all of samples studied display rather small negative Eu anomaly, which is considered to reflect that of a probably continental source material.

Two major theories, biogenic and volcanic, have been advocated to explain the genesis of cherty rocks. If volcanic activity is directly responsible for chert formation, the rocks so formed should not show a negative Ce anomaly. This preliminary investigation suggests that high-accuracy REE determinations can lead to useful results if applied to researches in siliceous sedimentary rocks and related subjects.

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Factors controlling grain weight in wheat

THE physiological factors controlling differences in grain weight between cultivars of wheat are not fully resolved. The experiments described here indicate that final grain weight in two cultivars of wheat is mainly dependent on the rate of accumulation of dry matter, which in turn is governed by the number of endosperm cells formed. Cell number in the endosperm seems to be regulated by the supply of assimilates available to the grain during the first 2 weeks after anthesis.

Two cultivars of wheat (*Triticum aestivum* L.), Maris Huntsman and Val, were grown in pots in the open from November 1975 to July 1976, and tillers were removed to maintain a constant density of 320 shoots per m^2. The plants reached anthesis on 7 and 8 June, Val being the earlier, and attained a mean grain number per inflorescence of 48.8 (Maris Huntsman) and 48.5 (Val). In the first experiment inflorescences were collected at intervals from anthesis to maturity. Grains were removed from the lower two florets of the 10 central spikelets of the inflorescence, and the weight per grain determined after drying to constant weight. The stage of development of the endosperm was determined from median transverse sections of grain fixed in gluteraldehyde, embedded in epon, and examined from 2-μm sections using phase contrast optics.

At maturity, Maris Huntsman contained 21% more dry matter in the grain than Val (Fig. 1), and the rates of increase in dry weight for the period 14–30 d after anthesis, calculated by regression analysis, were $1.63\pm0.09\ \mathrm{mg\ d^{-1}}$ (Maris Huntsman) and $1.12\pm0.07\ \mathrm{mg\ d^{-1}}$ (Val). The higher rate in Maris Huntsman could have been due to a greater supply of assimilate, or to its grain being a more efficient sink, or to a combination of the two factors.

It has been suggested[1] that the rate of increase in dry weight of the grain may be closely related to sink size as determined by the number of endosperm cells formed, and our data provide evidence for this. Examination of the developing endosperm showed that by 14 d after anthesis, the outer layer of endosperm cells had ceased to be meristematic in both cultivars, and had differentiated into recognisable aleurone cells, indicating that the number of endosperm cells had been fixed[2]. The effect of cell number in the endosperm on the rate of increase in dry weight and on final grain weight was tested in a second experiment, in which floret number per inflorescence was reduced to four on two dates by removing all florets except the basal floret of the four central spikelets. Florets were removed at

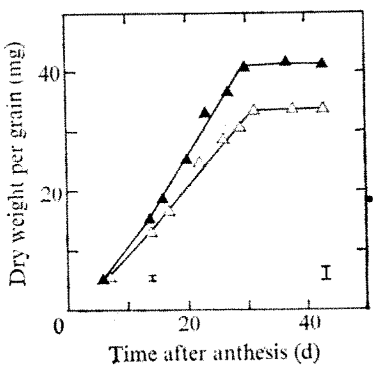

Fig. 1 Dry matter accumulation of the lower two grains of the 10 central spikelets of the inflorescence. Each point is the mean of 64 inflorescences. Vertical bars indicate the least significant differences ($P < 0.05$) at 15 d after anthesis and at maturity. ▲, Maris Huntsman; △, Val.

anthesis to provide an excess of assimilate available to the remaining grains during the period of cell division in the endosperm, and 15 d after anthesis, to give an excess of assimilate during the time when development was due entirely to cell expansion in the endosperm. Inflorescences were collected on three dates between 15 d and maturity, and the weight per grain was determined after drying to constant weight. The number of endosperm cells was determined in grains fixed in 1 : 3 acetic acid–absolute ethanol and macerated with fungal cellulase[3]. Grains from the basal floret of the four central spikelets in intact inflorescences were controls.

Reducing floret number at anthesis increased the dry weight per grain at 15 d and the number of endosperm cells in both cultivars, although the effect was larger in Val (Fig. 2, Table 1). Also, the rate of dry matter accumulation was stimulated, resulting in an increase in total dry weight at maturity of 13% in Maris Huntsman and 47% in Val. The reduction of floret number at 15 d produced only a small increase in the final dry weight of the grain.

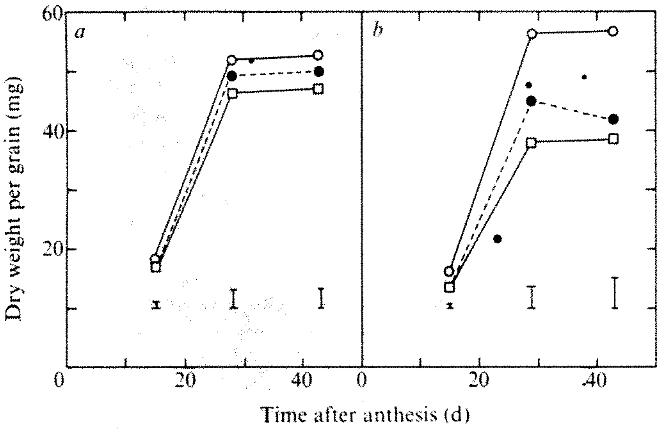

Fig. 2 Dry matter accumulation of the basal grain of the four central spikelets of the inflorescence. *a*, Maris Huntsman; *b*, Val. Each point is the mean of 20 inflorescences. Vertical bars indicate the least significant difference ($P \leq 0.05$) between the control and either treatment. □, Control; ○, florets removed at anthesis; ●, florets removed 15 d after anthesis.

Grain formed in intact inflorescences of Maris Huntsman contained 19% more endosperm cells and accumulated 21% more dry matter at maturity than those of Val. The difference in grain weight between the cultivars was closely correlated with the difference in the number of endosperm cells, suggesting that cell number in the endosperm is a major factor controlling the rate of increase in dry matter and final dry weight. The operation of this control is demonstrated by the small effect of increasing the supply of assimilate to the grain at 15 d, when the number of endosperm cells was fixed and further development was due to cell expansion only.

Grain weight in intact inflorescences seems to be determined mainly by differences in the supply of assimilate to the developing grains, which in turn regulates the size of the grain sink to match the amount of assimilate available.

The implications for plant breeding are twofold. First, the potential sink size in some wheat cultivars is a function of the number of cells in the endosperm and the level of assimilate supply during the meristematic phase of endosperm development. Second, the number of endosperm cells formed seems to be a reliable indicator for use in selection for the yield component, final grain weight.

Table 1 Effect of reducing floret number at anthesis on the number of endosperm cells formed per grain. Each value is the mean of 10 grains, 15 d after anthesis

	No. of cells	
	Control	Florets removed
Maris Huntsman	145,000	157,000
Val	122,000	158,000
s.e.m.	2,200	

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Pattern of lean and fat deposition in adults

THE fact that obesity becomes more common when societies change from a subsistence/labour intensive to an affluent/sedentary way of life might be taken simply as a demonstration that easy living encourages slothfulness and gluttony. Fat people, however, do not in general seem to eat more than thin ones, and there is increasing evidence of an inherited component of obesity. It seems more likely therefore that, throughout much of past history, individuals who had a genetic constitution predisposing them to store energy preferentially as fat have had some kind of advantage for survival. We have used a computer simulation model of energy balance to compare the effects of different seasonal patterns of work output and food availability on individuals with inherent tendencies towards either leanness or fatness, and to illustrate the circumstances and the manner in which the latter type has a relative advantage over the former.

In subsistence agricultural societies, most adults are smaller and take less food than those in affluent communities. The pattern of eating is often feast-and-fast; the heavy manual work of cultivating the next year's crops frequently coincides with reduced availability of food. In these conditions, the ability to store excess energy efficiently and to release it when needed for physical work has survival value. In affluent societies however, a regular, adequate food supply is guaranteed and activity is fairly constant throughout the year. There is not the same need for the body to store and release energy. Rather, people tend to overeat and longevity may be improved by the ability to 'burn-off' excess energy and so avoid obesity with its health penalties. A possible method of meeting these different requirements for optimal survival would be to vary the relative proportions of lean and fat tissue used in storing excess food energy. The efficiency of converting food to fat is about 70% (refs 1, 2); fat tissue has high energy density and low tissue maintenance requirements. Food energy is converted to protein with an efficiency of only 40% (refs 1–3); lean tissue has a low energy density and relatively high maintenance metabolic needs. Both fat and lean tissues are very efficiently mobilised for energy.

We have defined the ratio P as

$$P = \frac{\text{energy stored as (or mobilised from) protein}}{\text{total energy stored (or mobilised)}}$$

Values of P for non-obese subjects can be calculated from the classical starvation studies of Keys *et al.*[4], and Fig. 1 shows the distribution obtained.

The modal value of P is between 0.05 and 0.10, but a tail extends to much higher values. By contrast, in obese subjects the P values calculated from Passmore's starvation studies[5] were all very low and ranged from 0.02 to 0.04. In Keys's data there is a significant negative correlation between the value of P and the percentage of the body which was fat, but no correlation between the value of P and the total body weight. The P value is therefore associated with a body type but not a body

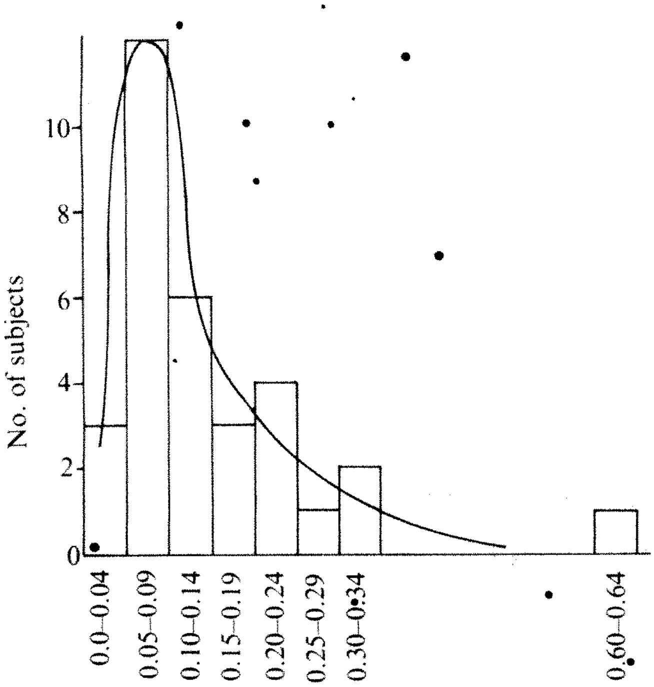

Fig. 1 A histogram showing distribution of values of the ratio of energy mobilised from protein to total energy mobilised. Taken from ref. 4.

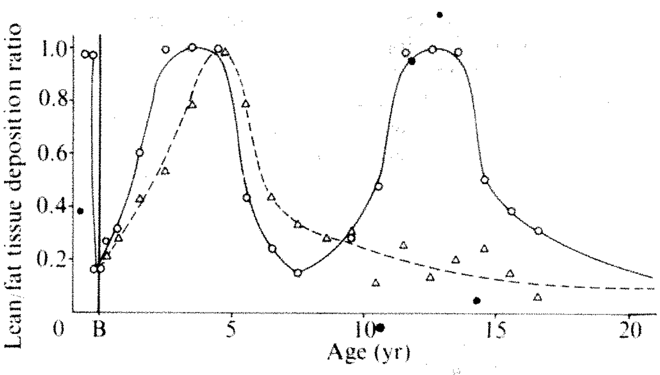

Fig. 2 Variations in children of the ratio calories deposited as protein to total energy deposited. ○, Boys; △, girls. Sources as given in ref. 6.

size. As the subjects in Keys's experiment were fasted, they lost most of their body fat but there was little change in P value. It seems likely that the value of P is set in an adult and remains fairly constant regardless of present body weight. In children, however, P varies widely with age and there are significant differences between boys and girls during adolescence[6] (Fig. 2).

The computer model[10] has enabled us to quantitate the effect of different P values among adults in subsistence and affluent societies. For both societies, we have modelled men with constant values of P set at $P = 0.03$ ('fat man') and at $P = 0.30$ ('lean man'). These are near the tenth and ninetieth percentiles of the range of P.

As an example of men in a subsistence community, we have used data from Gambian farmers with food intakes and activities at different times of the year as described by Fox[7]. The model has reproduced the observed weight changes (Table 1).

The total weight changes given by the model were similar in both 'fat' and 'lean' men. The 'fat man' used mainly fat to balance his energy budget, but the 'lean man' drew upon his lean tissue, which is largely muscle, to meet his energy deficits. The 'lean man' therefore depleted his muscles just when they were needed for heavy work. Although no field studies are available to us, it seems reasonable to suppose that the ability of an individual to perform sustained muscular work would be impaired by the loss of muscle tissue. In acute experiments[4] loss of handgrip power is correlated with loss of body lean tissue. The utilisation of fat as a storage medium therefore has two advantages; it is not only more efficient but also spares muscle tissue which is needed for manual work.

In affluent societies, body weight is slightly higher and maintenance energy needs are also slightly higher in consequence. In contrast to the Gambians, there are only minor seasonal variations in food intake and activity, and there is a tendency for intake to rise above maintenance needs. We have modelled the effects in the 'fat man' and the 'lean man' of 5% and 10% increases of intake above the requirement for weight stability. The mean food intake was kept constant at this new level and there was a 20% random variability about the mean intake to simulate daily variability[8] (Fig. 3).

The model predicts that body weight would rise and take 3–6 yr to reach new equilibrium levels. Table 2 shows that with a constant 10% excess of energy, the 'fat man' gained 18.1 kg in body weight, but the 'lean man' only 8 kg. More significant are the changes in body composition. In the 'fat man' the per cent body fat rose from 12% to 23%; the 'lean man' rose from 12% to 13%. Since the ill effects of obesity are probably related both to excess weight and excess fat, the 'fat man' may well suffer a double penalty for overeating the equivalent of one small chocolate bar a day.

In contrast to adults, the P values in children change during growth (Fig. 2). Using a modification of our computer model,

Table 1 Seasonal patterns of weight change and body composition for subsistence farmers, derived from computer model

'Fat man'	Total body weight, W (kg)	ΔW	Body fat, F (kg)	ΔF	Body lean L (kg)	ΔL	% Fat
Starting weight (59)	59.0		11.3		47.7		14
5 months cultivation		−4.0		−3.4		−0.6	
food ↑ work ↑ (55)	55.0		7.9		47.1		10
4 months harvest		+5.7		+4.8		+0.9	
food ↑ work ↓ (60)	60.7		12.7		48.0		15
3 months rest		−0.9		−0.8		−0.1	
food ↓ work ↓ (59)	59.8		11.9		47.9		14
'Lean man'							
Starting weight	59.0		11.3		47.2		14
5 months cultivation		−3.1		−0.9		−2.2	
food ↓ work ↑	55.9		10.4		45.5		13
4 months harvest		+4.8		+1.5		+3.3	
food ↑ work ↑	60.7		11.9		48.8		14
3 months rest		−1.3		−0.5		−0.8	
food ↓ work ↓	59.4		11.4		48.0		14

Observed weights[7] in parentheses.

we have shown[6] that a sustained shift up or down in the pattern of P values throughout childhood would produce substantial changes in body composition, equivalent to ectomorphy and endomorphy, even though food intakes were unchanged.

In adults it seems that the P value has implications for body composition, and in some circumstances, for body weight also. In subsistence communities it seems advantageous to use fat as the main energy store, and thus to spare muscle in conditions of stress. The low P value associated with this pattern of adaptation seldom produces obesity in these conditions because of the limited availability of food. As communities become more affluent, however, the survival advantages of a low P value no longer apply, as obesity and its complications become an increasing problem for the men with the low P values. The shape of the curve in Fig. 1 is compatible with a genetic basis for the distribution of P values. These data are derived from North American men, but have a low modal value, that is a 'fat man' value, consistent with adaptation to subsistence conditions. The presence of some individuals with a high P value might indicate genetic drift. Such a genetic mechanism could explain the inherited pattern of obesity commonly found in affluent countries. In affluent conditions, a genetic drift of the P value to higher levels would have no survival disadvantages and might even have positive advantages although these would occur mainly after reproductive age. If the affluent society persists, there may thus eventually be a higher proportion of 'lean' individuals.

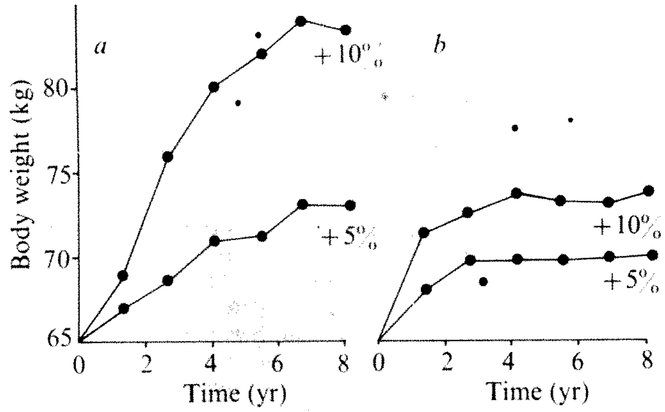

Fig. 3 Changes in body weight associated 5% and 10% above maintenance food intake. *a*, $P = 0.03$; *b*, $P = 0.30$.

James and Trayhurn[9] have speculated on the enzyme pathways which might be responsible for the utilisation of fat and protein to meet energy needs and we are developing a more sophisticated computer model to try to localise the critical pathways. The apparent importance of the P value in determining human body composition suggests that treatment of obesity could be effected by chemotherapy to raise the value of P as an alternative to present methods which stimulate the basal metabolic rate or depress appetite. If the P value could be raised from 0.03 to 0.30, then our model suggests that an obese man would slowly lose weight and fat without altering his energy intake.

Table 2 Computer model predictions of changes in body weight and composition with overfeeding of 'fat' and 'lean' men

	Total weight, W (kg)	ΔW	Fat tissue, F (kg) %	ΔF	Lean tissue, L (kg)	ΔL	
Starting values	65		11		54		12
Final levels with food intake 5% above maintenance requirements							
'Fat man'	73	+8.0	17.7	+6.7	55.3	+1.3	17
'Lean man'	68.9	+3.9	12.0	+1.0	56.9	+2.9	12.5
Final levels with food intake 10% above maintenance requirements							
'Fat man'	83.1	+18.1	26.2	+15.2	56.9	+2.9	23
'Lean man'	73.0	+8.0	13.1	+2.1	59.9	+5.9	13

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Dominance and reproductive success among female gelada baboons

THE relationship between dominance and reproductive success has been of considerable theoretical interest, with an extensive literature reporting its existence among males in many species[1]. Although female dominance hierarchies are known to exist in various species, the possibility of a similar relationship for females has not been investigated in detail. Indeed it is often assumed that Bateman's principle[2] implies that intrasexual selection (and hence the effectiveness of dominance as a reproductive strategy) will be very much reduced among females. Only one study of mammals presents unequivocal evidence of a relationship between dominance and reproductive success for females[3], although there is indirect evidence that it may occur in several other species[4-7]. We report here evidence for such a relationship in the case of female gelada baboons (*Theropithecus gelada*) in the wild.

Gelada baboons live in reproductive units that typically consist of a single adult male and up to 10 mature females with their offspring[8]. During a field study of this species in Ethiopia in 1974–5, dominance ranks among the females in each of 11 reproductive units were determined by analysing the frequencies of approach–withdrawal type agonistic interactions within each unit. In all cases, the hierarchies were found to be strictly linear.

The number of immature animals less than 3.5 yr old at the start of the study was known for each unit, together with the number of infants born into each unit during the subsequent 9 months of intensive study. From detailed studies of the social structure of these units, it was possible to determine probable mother–offspring relationships with some confidence. Survivorship for immature animals up to the age of 3.5 yr was estimated to be 80% (unpublished data). Thus, on average, the data for each unit will deviate from either the true number of births or the number of offspring surviving to the age of 3.5 yr by only about 10%. In view of this, no attempt was made to correct the raw data in any way.

Figure 1 shows the mean number of offspring per female for females of the various dominance ranks. There is a significant correlation between these two variables ($r_s = 0.667$, $n = 8$, $P < 0.05$), and a linear function has been fitted to the data by the method of least squares. In practice, a linear function is *a priori* unlikely, because, no matter how low a female's dominance rank may be, she will have some finite (albeit small) probability of reproducing. But, fitted exponential and hyperbolic functions give only marginal increases in goodness of fit.

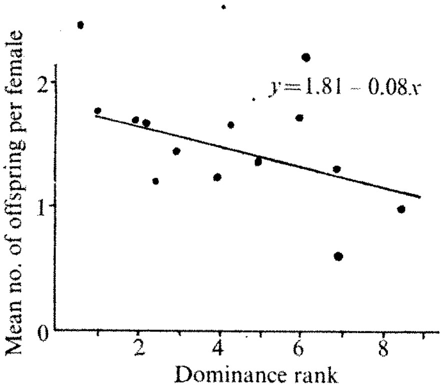


Fig. 1 Relationship between the mean number of offspring per female and rank in the female dominance hierarchy (see text for details).

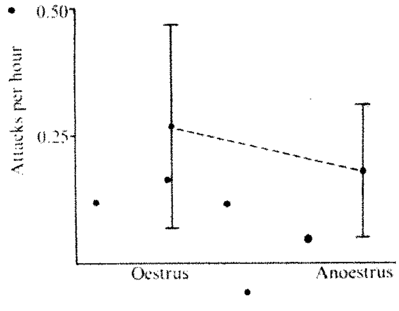
Since the linear function does not yield a zero value for reproductive output until the equivalent of rank 23 (a value well beyond the normal range of unit size for this species[8]), a linear approximation is quite adequate for most purposes.

Two alternative explanations for these results might lie in a confounding of variables. First, it could be argued that, as the number of females in a unit increases, mutual interference among them might result in a lowering of each of their individual reproductive rates by the same amount. The fact that the samples for low ranks are based on large units, whereas those for high ranks are based on both small and large units, will tend to bias the results in favour of the latter. Consequently, the average output would seem to be higher for dominant females than for subordinates, although there was no underlying relationship between dominance and reproductive success. That this is not so in the present case can be shown by comparing the reproductive outputs of the top three ranking females in small units (four or five females) with those of the top three females in large units (six to nine females). The former averaged 1.889 offspring per female, compared with 1.778 for the latter: the difference is not significant (randomisation test: $t=0.509$, d.f.$=16$, $P > 0.0$). Second, it could be argued that, because the number of offspring a female can expect to have will be in part a function of her age, the results obtained could be due to a relationship between age and dominance rank. It can be shown, however, that there is no significant relationship between these two variables; furthermore, it can also be shown that, while there is a relationship between age and number of offspring, this is independent of the relationship between dominance and number of offspring (unpublished data).

The available evidence suggests that dominant females achieve this reproductive differential by harassing subordinate females, thereby causing them to undergo more oestrous cycles before conception than do dominants. This can be seen from two relevant sets of data.

First, Fig. 2 compares the frequencies with which a sample of nine females were harassed by females in their units who were more dominant to them when they were in oestrus and when they were out of oestrus. For these purposes, oestrus is defined as the part of the menstrual cycle during which the areas of sexual skin are surrounded by fluid-filled vesicles, sexual activity being most common during this period[9,10]. The difference between the two conditions is significant (randomisation test for matched pairs: $P=0.023$, $n=9$), attacks being about 50% more common during oestrus than during anoestrus.

Fig. 2 Mean frequency of attacks received from the more dominant females of their units by females who were in the oestrous and anoestrous parts of the menstrual cycle (vertical bar gives 1 s.d. either side of the mean).



Second, an examination of (1) the order in which sets of females which were synchronised in their menstrual cycles stopped cycling and (2) the order in which sets of females who gave birth within a period of 2 months of each other did so indicates that the order of (probable) conception corresponds closely to the order of dominance ranks. To maintain statistical independence, the data were analysed by considering only successive dyads in each set: in other words, triads, for example, were treated as two sets of successive dyads, *A*-with-*B* and *B*-with-*C*. On this basis, there were nine dyads in each of the two data groups, of which only two dyads in each case were in reverse order of dominance (binomial tests: $P=0.09$ in each case, $n=9$). Combining probabilities by the method due to Fisher[11] gives $\chi^2=9.632$, which, with $2k=4$ degrees of freedom, is significant ($P < 0.05$), indicating that order of conception tends to follow order of dominance ranks.

The precise *modus operandi* of this process is uncertain, but suspicion rests strongly on the likelihood that harassment during oestrus results in a disruption of the female's ovulatory cycle, either by causing it to be anovulatory or by precipitating spontaneous abortion of the ovum before or soon after fertilisation. Two lines of evidence support this interpretation. First, no differences could be found between dominant and subordinate females in either the rates of mounting or the frequencies of ejaculation received from their males. Furthermore, no female was ever observed to actively interfere with the solicitations or copulations of other females. Second, there is circumstantial evidence from captive studies of both *Theropithecus* and *Papio* that intense aggression and/or stress can result in the sudden, premature termination of oestrous cycles[10,12].

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Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae)

IF offered a choice of cabbage or *Reseda* the large white butterfly (*Pieris brassicae*) first lays a certain number of eggs on the former food-plant and then oviposits on ***Reseda***. We have found that several cues enable the butterfly to 'assess' the egg load present on a plant or leaf, and at the appropriate moment switch to the previously less acceptable species. Overloading is thus avoided. Feeding larvae and damaged foliage are also deterrents to oviposition.

Egg-laying females (of the Cambridge and Oxford strain of *P. brassicae*[1]), confined in net-covered cages, were offered a choice of two cabbage leaves from the same plant, matched for age, position on the stem, size, shape and texture. Damaged leaves and those bearing signs of mildew, aphid or slug infestation were discarded. One whole plant (with a control) was offered on which *P. rapae* had oviposited. The types of leaves used, with their treatment, are shown in Table 1. To avoid position effects, leaves were transposed periodically.

We found that *P. brassicae* lay a reduced number of eggs on leaves bearing their own eggs; model plastic eggs; crushed egg fluid; body fluids pressed from the tips of female abdomens; feeding caterpillars; and leaves from which egg batches had been removed. They were not inhibited by larvae placed on a leaf surface, by model caterpillars or by an airstream with larval odour. The most powerful deterrents were feeding larvae and certain plant extracts[2] (Tables 1 and 2).

The butterfly's response to yellow plastic eggs or yellow patches painted on the leaves (a single trial) indicated that visual cues contribute to the selection of suitable leaves. Similarly the repellant effect of the eggs was reduced if they were dyed green. Vision may also play a part in the rejection of leaves on which larvae are feeding or which display old damage, holes and emarginations[3].

The reduction in number of aerial visits (without leaf contacts) to plants and leaves bearing hidden eggs, crushed egg fluid or body fluids, or from which eggs had been removed, indicates that airborne cues associated with the presence of eggs are appreciated from a distance.

Similar results were obtained with recently damaged leaves and crushed leaf extract painted on the surface. The reduction in the number of settlings after contact with experimental leaves carrying a heavy egg-load, or smeared with egg fluid (Table 2), indicates that chemoreception through the tarsi[4] also occurs.

The relative increase in the number of small egg batches (1–10) and the associated paucity of large batches (60 upwards) and a lack of orderly linear arrangement of eggs on experimental leaves, suggests that the female experiences disturbing or discouraging elements while in the act of laying, some of which may be perceived through the tip of the abdomen. This was also intimated by the behaviour of females attempting to lay on paper leaves (Table 2).

The colour and shape of eggs, if visible to the approaching butterfly (Table 1) represent one aspect of the deterrent, but our experiments indicate that an airborne pheromone-like emanation associated with the egg is the most important factor discouraging the fluttering female. After the removal of egg batches, leaves could still inhibit oviposition (Table 1), suggesting that the female, while laying, adds a deterrent marker to the eggs, either incorporated in the cement (which is difficult to remove from the leaf surface) or consisting of a discrete secretion of the accessory glands associated with the reproductive organs or genitalia, or the ovaries themselves.

Experiments using the small white (*Pieris rapae*) and

Table 1 *P. brassicae* ovipositing in experimental situations on cabbage leaves

Condition of leaf	No. of trials	No. of eggs trials	Remarks
Egg laden	45	1,129	13 leaves without eggs; none with > 150 eggs
Clean		6,913	No leaves without eggs; 16 with > 150 eggs
Smeared with *P. brassicae* egg fluid	6	317 (7 batches)	3 leaves without eggs
Smeared with distilled water		1,410 (34 batches)	No leaves without eggs
P. brassicae eggs brushed off surface	11	858 (25 batches)	1 leaf with under 10 eggs; none with 150 eggs
Surface brushed only		3,199 (93 batches)	All leaves with over 10 eggs; 1 leaf with > 150 eggs
With yellow model eggs attached	9	70 (4 batches)	The ♀ sometimes investigates model egg batches by extending the proboscis and drawing it back and forth over the surface like a bow across a violin
Clean		750 (68 batches)	
With first instar larvae feeding	4	140 (14 batches)	
Clean (treated with crushed cabbage extract in 2 trials)		750 (68 batches)	Contrast would have been greater on a clean leaf without extract
Damaged by feeding larvae	8	628 (18 batches)	
Undamaged		2,663 (74 batches)	
Smeared with tip of ♀ abdomen	8	234 (15 batches)	The leaf may have also been contaminated with egg fluid, haemolymph, mecomium and fluid from the gut
Smeared with distilled water		2,880 (67 batches)	
Red cabbage (red drumhead cultivar)	2	40 (3 batches)	
Green cabbage (greyhound cultivar)		2,008 (58 batches)	
White (heart) leaf from same plant as control	2	313 (13 batches)	Larvae only very rarely metamorphose when reared on cabbage 'hearts'
Green leaf (flower of spring cultivar)		2,658 (84 batches)	
Old (growing)	3	874 (19 batches)	This also applies if whole young plants are matched against older plants
Young (growing)		1,008 (42 batches)	

the monarch (*Danaus plexippus*) both of which lay eggs singly, suggest that a similar mechanism in these butterflies limits overloading of the host plant (Table 3). Whereas the large white chiefly depends on tarsal contact for testing the suitability of the leaf, the small white uses the tip of the abdomen, while the ovipositing monarch generally taps the leaf surface with the antennae after settling, although vision seems to have a role where larval infestation is concerned. W. W. Brandt (personal communication) has seen birdwings (Ornithoptera) in New Guinea apparently searching *Aristolochia* vines visually (starting from the bottom and working upwards) and flying off should they discover the presence of an egg on a leaf. If such an egg is removed, the next female searcher will lay on the plant.

Table 2 Behaviour of ovipositing *P. brassicae* and evidence of airborne and contact cues

Cabbage leaf treated with plant extracts or egg material	No. and duration (h) of trial	Aerial visit without contact	Visits with leaf contacts*	Settling on leaf (wings folded but no oviposition)	No. of eggs
Repellent plant extract (5 species tested)	7 trials	125	27	15	136
Cabbage leaf extract	8	243	47	48	812 (+14 batches NC)
Attractant plant extract (3 species tested)	3 trials	16	35	23	271 (+9 batches NC)
Cabbage leaf extract	5	18	41	21	175 (+9 batches NC)
Cheiranthus extract (containing attractant and repellent)	10 trials	59	NC	NC	26
Cabbage leaf extract	10	168	NC	NC	602
P. brassicae eggs on cabbage leaf	1 trial	NC	8	Nil	Nil
Clean cabbage leaf	1	NC	33	3	2 egg batches
Crushed *P. brassicae* eggs on upper surface of cabbage leaf	2 trials	24	NC	NC	2 egg batches
Cabbage leaf extract	2	48	NC	NC	6 egg batches
P. brassicae eggs brushed off surface of cabbage leaf	2 trials	NC	14	2	30 (2 batches)
Cabbage leaf (surface brushed)	3	NC	11	Nil	270 (14 batches)
P. brassicae eggs removed from surface of cabbage leaf	5 trials	38	NC	62	893 (25 batches)
Clean leaf (surface brushed)	13	43	NC	116	2,366 (76 batches)
Green paper leaf + distilled water on surface	2 trials	69	NC	NC	Nil
Green paper leaf + cabbage leaf extract	2	82	NC	NC	4 ♀♀ ovipositing† 1 batch

NC, not counted.

*The leaf was touched with the feet or wings but fluttering continued until the butterfly flew away.

†These females went through all the motions of laying egg batches but only in one case actually extruded eggs. The small white butterfly (*P. rapae*) regularly tests the leaf with the tip of the abdomen and then decides whether or not to lay, but this is unusual for *P. brassicae*.

Similar undetected deterrents associated with eggs and feeding larvae may be widespread among the Lepidoptera. This could explain the development by some plants, such as *Passiflora*, of faked eggs[3] and larva-like stipules[5]. Such a response to heavy butterfly predation assumes visual assessment by the laying female. It would be interesting to investigate the scent of these plant-part mimics, especially the faked eggs.

Repellent markers have not been recorded in the Lepidoptera before, but have been described in entomophagous parasites as a means by which the female prevents overparasitisation of the host[6]. A chemical associated with the eggs of the noctuid moth *Heliothis virescens*, present on, in and around the eggs after oviposition and in ovarial tissue (but not in accessory gland extract) is said to elicit host area searching in an egg–larval parasitoid[7]. In the case of the cherry fruit fly (*Rhagoletis cerasi*) and related species[8,9], marking of the host plant is affected by active spreading of the repellant across the fruit surface by the ovipositor. In *P. brassicae* it seems that the pheromone-like repellent is excreted on to the leaves or, as in *Heliothis*, is present in the tissue or covering of the egg itself.

It was not surprising that *P. brassicae* responded more positively to feeding larvae than to its own eggs, since such a threat to the food source is immediate and certain. It was also to be expected that deterrents found in lethal food plants (for example, *Cheiranthus cheiri* (wallflower) and *Erysimum* (treacle mustards) (refs 2 and 10 and unpublished results)) which also contain the usual cruciferous oviposition and feeding stimulants, would be both powerful and decisive in their action. Moreover, the quality of the deterrent is marvellously suited to the large white butterfly's lifestyle, for it neither inhibits nor reduces egg laying too drastically. By urging the female, fluttering among the cabbages, to 'try her luck' just a little further on, it ensures the dissemination of her progeny on suitable foliage.

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Table 3 Experiments with ovipositing *P. rapae* and *D. plexippus*

Alternatives offered to *P. rapae*	Duration of experiment	No. of eggs	Remarks
Leaf of green cabbage (greyhound)	4 trials 1 h	105	—
Leaf of red cabbage (red drumhead)		12	
Cabbage plant in pot	12 h*	254	
Tropaeolum plant in pot		196	Eggs also laid on flowers
11-leaved clean cabbage plant	12 h*	245	—
13-leaved egg-laden cabbage plant		129	—
Single clean cabbage leaf	(*a*) 4 trials 30 min	(*a*) (*b*) 60 187	
Single egg-laden cabbage leaf	(*b*) 1 trial 3 h	(*a*) (*b*) 14 116	
Clean cabbage leaf	2 h	86	52 contact visits
Cabbage leaf with 80 eggs crushed on underside		21	25 contact visits
Clean cabbage leaf	1 h	46	—
Leaf with batches of *P. brassicae* eggs crushed on surface		20	—
Clean cabbage leaf (underside brushed)	1 trial 15 min	66	♀♀ very keen to lay and clean leaf became overcrowded
Cabbage leaf with 12 ♂♂ abdomens lightly brushed on underside		45	
Small clean cabbage leaf (lightly brushed on underside)	3 trials 40 min	133	
Small leaf from which 90 eggs were lightly brushed off underside		53	
Alternatives offered to *D. plexippus*			
A. curassavica clean plant	3 trials 30 h	281	♀♀ near end of egg laying
A. curassavica egg-laden		243	
A. curassavica clean plant	3 trials 12 h	112	♀♀ near end of egg laying
A. curassavica with feeding larvae		40	

In the absence of leaves, *P. rapae* will oviposit on objects in the cage whereas *P. brassicae* dies without laying eggs.

*When the favoured leaf becomes egg laden the contrast declines.

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Tumours and viruses in mice injected with plutonium

THE α-particle emitting plutonium is, at present, the bone-seeking element of greatest public concern. For man, however, the best evidence of damage from α-particle emitters in bone concerns radium, for which the pattern of deposition differs from that of plutonium. We report here an experiment designed to compare the effects of plutonium in mice with those of radium, previously reported[1,2].

The specific questions we considered were (*a*) Do plutonium-induced tumours contain viruses? (*b*) Does sex affect the viral status or incidence of tumours? (*c*) Are the tumours similar to those induced by radium? (*d*) Are they variable with dose and time, or different irradiation chimaeras[3].

The solution injected intraperitoneally was minimally polymerised plutonium (predominantly ^{239}Pu) citrate[4] passed through a 25-nm Millipore filter.

The design of the experiment and distribution of tumours in each of eight separate groups of animals are shown in Fig. 1. No controls are included in Fig. 1, but for 15 CBA/H female controls carefully examined earlier, and hundreds of normal CBA/H mice maintained until death but less rigorously examined, no osteosarcomas were observed. The median lifespans of untreated male and female mice are about 24–28 months beyond the age of 3 months, when treated mice were injected. These figures differ little from those of CA/H mice injected with 10 nCi ^{239}Pu (22 and 26½ months, respectively; Fig. 1).

In chimaeras CBA *T6T6*/A, 9/20 males and 14/20 females developed osteosarcoma after 100 nCi ^{239}Pu. Genetic tests by passage[3] of these tumours and one skeletal haemangiosarcoma confirmed that they originated from host tissue.

In natural mice given 100 nCi, leukaemias were observed in the same number of animals as osteosarcomas, 2/10 males and 5/10 females. After 30 nCi, osteosarcoma occurred in 4/10 male and 8/10 female mice. After 10 nCi, no osteosarcomas were observed in male mice but 9/10 died of other tumours including four carcinomas, two fibrosarcomas, two reticulum-cell sarcomas and one possible haemangiosarcoma: in females, however, osteosarcoma was the commonest tumour (6/10), accompanied by carcinoma in three, and two mice died early with reticulum-cell sarcoma.

Osteosarcoma is more readily induced in female than male mice, owing to the response of murine bone to oestrogens[5] (though in man the natural incidence of osteosarcoma is higher in males than females[6,7]).

The high incidence of tumours other than osteosarcoma, though unexpected, could agree with observations for rats inhaling soluble ^{238}Pu (ref. 8) and rats injected with various bone-seeking radionuclides[9]. Regarding leukaemia, though a small incidence of myeloid leukaemia[10] and "leukoses of the reticulosis type"[9] occurred in rats given plutonium, it has not been reported in mice. In CBA with a natural incidence of about 1% (refs 11, 12), seven cases out of 20 at risk of generalised lymphomatosis (excluding thymus) with lymphoblasts in peripheral blood was striking. Leukaemias occurred earlier than osteosarcomas. Also, there is a suggestion of an increase of early reticulum-cell sarcoma in animals given 10 nCi (the incidence in normal CBA mice is 5–10% (refs 2, 13) and of miscellaneous malignancies after 10 and 30 nCi, many at times later than the mean time for appearance of osteosarcoma (and so reminiscent of the late onset of specific carcinomas of cranial air sinuses in human radium cases, notably those with lower burdens of radium[14]). The late onset may be related to the long retention of radioactivity and immunological imbalance.

None of the chimaeras, which have perhaps an even lower

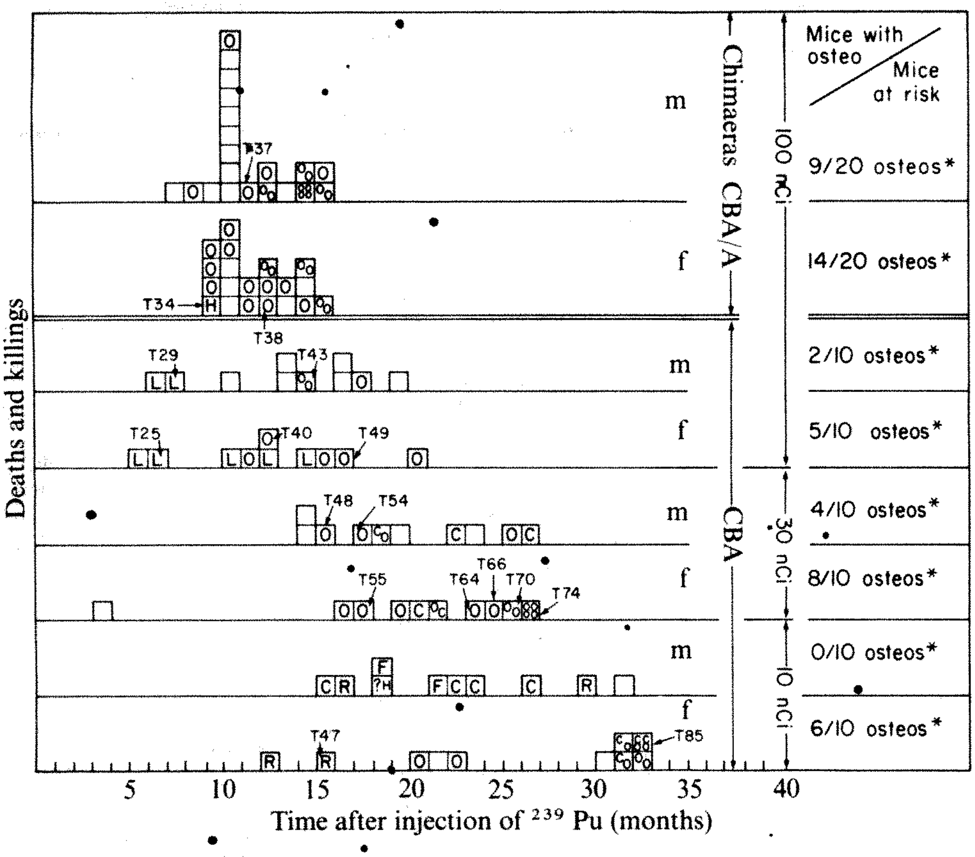

Fig. 1. Design and results of the experiment involving i.p. injection of CBA/H mice and CBA *T6T6*/A radiation chimaeras with ^{239}Pu citrate. Each mouse is represented by an individual box. This is plotted with time after injection of varying doses of ^{239}Pu citrate when the mouse either died or was killed. The types of tumours are indicated. O, osteosarcoma; H, haemangiosarcoma; L, leukaemia; C, carcinoma; F, fibrosarcoma; R, reticulum-cell sarcoma. Multiple entries indicate multiple tumours. Samples examined by electron microscopy are designated by the letter T followed by a number, for instance, T37. *osteo, Osteosarcoma; m, male; f, female.

incidence of all forms of lymphoma[15] than natural mice, developed leukaemia after 100 nCi. This may be due to a somewhat lower uptake of plutonium by bone marrow in chimaeras[16]. Such chimaeras, however, have a low incidence (1/69) of generalised lymphoma induced by β rays of ^{90}Sr–^{90}Y on donated haematopoietic tissue[17]. One might expect the 1,000 rad of whole-body irradiation given to the chimaeras to alter the normal cellular and humoral environment, but not to impair the expression of virus, rather the reverse[18].

The median lifespans of animals treated with 10 nCi (24 months) 30 nCi (20 months) and 100 nCi (13 months for CBA/H mice and 11 months for chimaeras) were shorter than for untreated controls (26 months). Malignant disease, varying in type between groups, was a major cause.

Table 1 lists the viral status of tumours examined electron micrographically. In osteosarcomas taken from chimaeras injected with 100 nCi ^{239}Pu, more virus particles were seen than in the other tumours. In 16 out of 19 tumours examined, virus was seen, and 'virus-like' particles were seen in one other tumour. No virus was observed in either of two tumours examined from animal T85. Figure 2 is an electron micrograph demonstrating the typical type-C particles. Viruses were commonly found either lying within cytoplasmic vesicles or budding from endoplasmic reticulum associated with degenerate cells. No difference could be seen in morphology of viruses in samples taken from different tumour types.

In the series no tissue taken from normal ribs of three CBA/H tumour-bearing animals showed any evidence of virus even after extensive search, though occasional viruses were seen in control animals of an earlier study[1].

Table 1 Viral status of ^{239}Pu-induced tumours

Dose per mouse (nCi)	Strain	Sex	ANL no.	Tumour type	Site of tumour	Time of appearance (months)	Virus present or absent
100	Chimaeras CBA *T6.T6*/A	Male	T37	Osteosarcoma	Sacrum	11	+
		Female	T34	Haemangiosarcoma	Sacrum	9	+
			T38	Osteosarcoma	Sacrum	12	+
	CBA	Male	T29	Lymphoma	Lymph node	7	+
			T43	Osteosarcoma	Right ilium	14	+
				Osteosarcoma	Thoracic vertebra 12		+
		Female	T25	Lymphoma	Lymph node	6	– ?
			T40	Osteosarcoma	Ischio pubis	12	+
			T49	Osteosarcoma	Humerus	16	+
30	CBA	Male	T48	Osteosarcoma	Lumbar vertebra 5	15	+
			T54	Osteosarcoma	Patella	17	+
		Female	T55	Osteosarcoma	Parosteal to humerus	17	+
			T64	Osteosarcoma	Cranium	23	+
			T66	Osteosarcoma	Sacrum	24	+
			T70	Osteosarcoma	Sacrum	25	+
			T74	Osteosarcoma	Scapula	26	+
10	CBA	Female	T47	Reticulum-cell sarcoma	Liver	15	+
			T85	Osteosarcoma	Rib	32	–
				Carcinoma	Lung		–

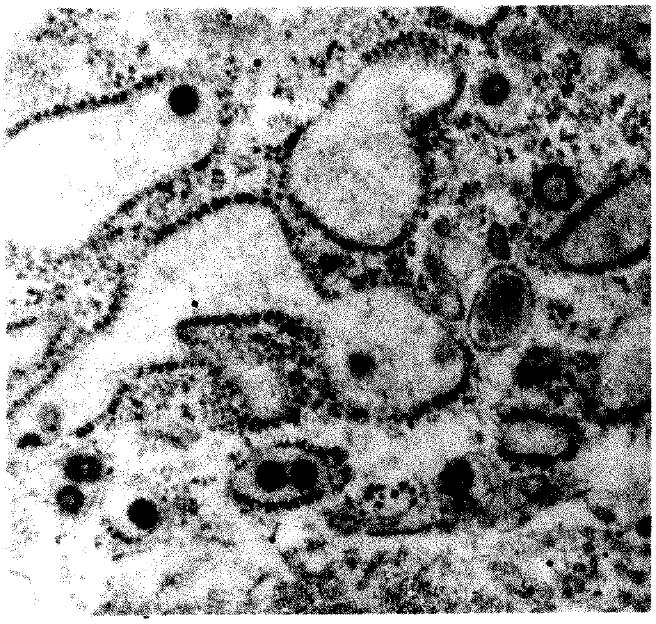

Fig. 2 Electron micrograph of a portion of tissue taken from an osteosarcoma (T54) showing viruses lying within cytoplasmic vesicles or budding from the endoplasmic reticulum (×47,600).

The particles appear identical with those observed in osteosarcomas from CBA/H animals injected with ^{226}Ra (ref. 1). This does not allow us, however, to implicate virus as the aetiological agent. Tumour tissue may merely provide a favourable environment in which virus can replicate sufficiently to be above the threshold of detection by electron microscopy. Three lines of evidence from current work in progress indicate that viral action need not be involved in radiation-related tumorigenesis. First, animals subjected to a procedure of immunisation with homogenates of tumour tissue and subsequently injected with ^{226}Ra develop osteosarcoma to about the same extent and in the same time as unimmunised controls[19]. Second, a single α particle directly hitting the nucleus may be insufficient to kill the cell[20], contrary to what some have previously supposed. Hence, survival of some cells with alteration of DNA is probable after a direct hit on the nucleus, and may lead to malignancy without the intervention of virus. Third, cells irradiated in culture with α particles have been transformed[21], in a manner similar to that shown by other workers using γ rays[22] and chemical agents[23], where transformation was shown to be independent of virus expression.

Our results need confirmation with larger groups of animals and smaller doses of plutonium (the lowest dose used led to a concentration about a thousandfold greater than that occupationally permissible to man).

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RD-114 and feline leukaemia virus genome expression in natural lymphomas of domestic cats

THE widespread distribution of endogenous type C viral genetic sequences in many different vertebrate species[1,2] is now well established. The biologic implications of the presence of such endogenous type C viral genomes, especially with respect to normal and neoplastic growth, are at present of considerable interest[3]. The domestic cat (*Felis catus*) offers a unique model system for approaching this question, because of its ready availability, outbred nature and relatively short lifespan. Moreover, the genetic information of its endogenous xenotropic virus (RD-114) is distinct[4] from that of feline leukaemia virus (FeLV), a horizontally transmitted, infectious type-C virus of this species. RD-114 virus[5] is generally non-infectious for cat cells[6] and its pathogenicity is undetermined, whereas FeLV is known to be leukaemogenic under natural[7] and experimental[8] circumstances. The genetic information of RD-114 has been demonstrated in the DNA of domestic cats[9–11] and their close relatives, but not in more distantly related species of the genus *Felis*[12]. Both transcriptional[13] and translational[14] products of the endogenous RD-114 viral genome are also found in certain instances in the absence of complete virus in normal cat tissues. FeLV and RD-114 gene expression at the transcriptional and translational levels in domestic cats has been examined here in relation to naturally occurring lymphoma. Results indicate a significantly higher level of RD-114 transcription and translation in most lymphoma tissues compared with normal tissues. Although FeLV expression is also pronounced in lymphomas, this generally reflects the presence of infectious FeLV. In general, however, only the lymphomas from the younger cats show significant levels of FeLV gene expression, whereas RD-114 expression is high in lymphomas of both young and old cats. Although the relationship of this enhanced expression of the RD-114 genes to the neoplastic process is unknown, these findings indicate that certain functions of the endogenous RD-114 viral genome are closely associated with the development of lymphoid tumours in the cat.

Nucleic acid and antigenic differences between RD-114 and FeLV made possible the measurement of the expression of viral information for both viruses in the same tissues at the RNA and structural protein levels. The transcription of virus-specific RNA in the tissues was determined by molecular hybridisation using ^{3}H-labelled single-stranded DNA products (^{3}H-cDNA) complementary to viral nucleotide sequences and the S_1 nuclease method of assay. The levels of the 30,000 molecular weight major structural protein (p30) of both viruses were determined on the same tissues by competition radioimmunoassays. Sixty histologically normal tissues from 47 cats free from cancer and 55 lymphoma tissues from 31 lymphomatous cats were assayed for the RNA and p30 of RD-114 and FeLV. The normal tissues examined included spleen, liver, and

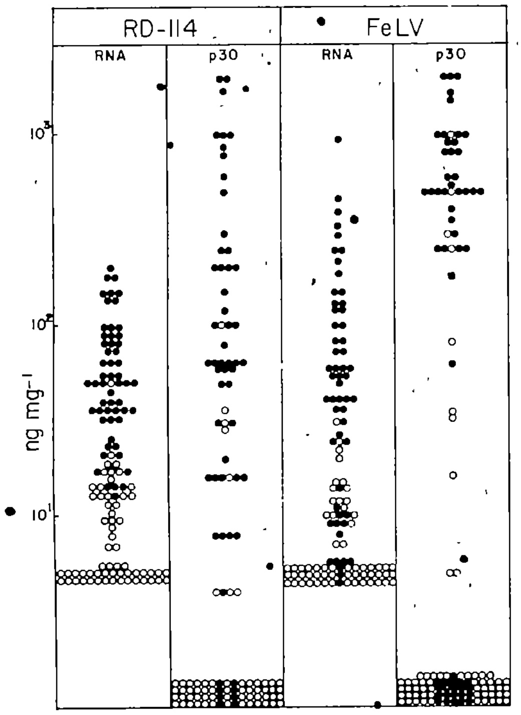


Fig. 1 Measurement of RD-114- and FeLV-specific RNA and p30 in normal and lymphoma tissues of cat. Viral RNA concentrations were determined by molecular hybridisation of tissue RNA with viral ^{3}H-cDNA using the S_1 nuclease assay. Total tissue RNA was prepared following a modification of a published procedure[20]. ^{3}H-cDNA was synthesised using a modified procedure of Benveniste and Scolnick[21]. Briefly, purified[22] RD-114 virus or FeLV (Gardner–Arnstein strain) grown in the human rhabdomyosarcoma (RD) cell line[4] was incubated at 37 °C for 4 h in a reaction mixture containing 0.024 mM ^{3}H-dTTP (92.9 Ci mmol^{-1}), 3 mM each of dATP, dGTP, dCTP, 20 μg ml^{-1} actinomycin D, 60 mM KCl, 6 mM $Mg(CH_3COO)_2$, 5 mM dithiothreitol, 40 mM Tris-HCl, pH 8.0, and 0.02% Nonidet P-40. ^{3}H-cDNA was shown to contain representative transcripts by its ability to protect ^{32}P-labelled viral RNA from digestion by RNase A and T_1 (ref. 23). RD-114 ^{3}H-cDNA hybridised with 78% of the viral RNA at a ^{3}H-cDNA to ^{32}P-viral RNA ratio of 1.0. FeLV ^{3}H-cDNA protected 67% of FeLV viral RNA at the same ratio. The viral RNAs were prepared as described previously[24]. The specificity of the probe was shown by the ability of RD-114 cell and viral RNA to protect 95% and 100%, respectively of the RD-114 ^{3}H-cDNA from S_1 nuclease digestion, whereas only 3% and 2% of the probe was protected by FeLV viral- and FeLV-infected RD cell RNA. FeLV ^{3}H-cDNA hybridised at 51% and 56% with homologous infected cell RNA and viral RNA, respectively, whereas only 2% and 5% of the ^{3}H-cDNA hybridised to heterologous RD-114 cell and viral RNA. All hybridisation reactions were carried out in 10 μl containing 0.3 M NaCl, 0.03 M Na citrate, 0.05% SDS, 0.02% diethylpyrocarbonate, 500–800 c.p.m. ^{3}H-cDNA (7.7×10^7 c.p.m. μg^{-1}) and various amounts of tissue RNA in sealed capillary tubes at 68 °C for 16 h. Hybridised fractions of ^{3}H-cDNA were determined by TCA-precipitable radioactivity after digestion with 35 μg ml^{-1} S_1 nuclease in a reaction mixture containing 0.17 M ZnCl, 27 mM Na acetate (pH 4.5), 0.11 M NaCl and 17 μg ml^{-1} heat-denatured salmon sperm DNA for 70 min at 45 °C. Concentration of RNA × time of incubation (C_rt) values up to about 3×10^4 (corrected to a monovalent cation concentration of 0.18 M; ref. 25) were generated with tissue RNA and the concentration of virus-specific RNA per mg cell RNA was determined by comparison of the C_rt value at 20% level of hybridisation with that obtained using viral RNA. The lower limit of sensitivity varied between 5–10 ng virus-specific RNA per mg cell RNA, depending on the amount of tissue RNA available for the assays. Tissues with undetected levels of RNA were arbitrarily listed as 5 ng per mg cell RNA. The thermal denaturation of hybrids formed between the RNA from two tumour tissues and the ^{3}H-cDNA probes of RD-114 and FeLV was determined for comparison with the corresponding T_m values obtained with RD-114- and FeLV-infected cell RNA. The hybrids exhibited a similar T_m (81.5 °C in 0.3 M NaCl, 0.03 M Na citrate, 0.05% SDS), indicating that most of the viral RNA generated in the tissues was closely related to the RNA produced in the virus-infected cell cultures. The values (ng per mg cell protein) of p30 were determined by group-specific competition radioimmunoassays using ^{125}I-labelled p30 antigen of FeLV and RD-114 as probes[26]. Cell extracts were prepared by homogenisation of tissues in equal volumes of 0.01 M Tris-HCl, pH 7.8, 0.1 M NaCl, 0.5 mM EDTA and 0.5% Triton X-100, sonication for 1 min by use of a Biosonic II sonic oscillator, and clarification by centrifugation for 1 h at 100,000g. Antisera prepared in goats to either FeLV or RD-114 and serial twofold dilutions of competing tissue extract were incubated at 37 °C in 0.15-ml reaction mixtures containing 10 mM Tris-HCl, pH 7.8, 10 mM EDTA, 0.4% Triton X-100, 1% bovine serum albumin, 0.1% normal goat serum, 10 mM NaCl, and 300 μg ml^{-1} phenylmethane sulphonyl fluoride. About 10,000 c.p.m. ^{125}I-labelled FeLV or RD-114 p30 in 0.05 ml of the same buffer were added and incubation continued for 3 h at 37 °C and a further 18 h at 4 °C. Antigen–antibody complexes were precipitated by the second antibody technique and radioactivity in the precipitates measured in a Searle model 1285 gamma counter. Levels of p30 in extracts, expressed as ng per mg cell protein, were calculated on the basis of displacement of the competition curve for the unknown relative to that achieved with purified viral antigen. Cell protein determinations were performed according to the method of Lowry[27]. The lower limit of sensitivity of the competition radioimmunoassay was 2 ng per mg cell protein. Tissues with undetectable levels of p30 were arbitrarily listed as 1 ng per mg of cell protein. ○, Histologically normal tissues from cats free of cancer; ●, lymphoma tissues.

kidney; most of the lymphomas were located in the spleen, lymph nodes, kidney, and a few were in the liver, thymus, and other tissues. The presence of infectious FeLV was determined in each cat by the indirect immunofluorescent antibody test for cytoplasmic FeLV p30 antigen in leukocytes and platelets of fixed peripheral blood or bone marrow smears. This test has proved to be a reliable index of systemic infection with FeLV (ref. 15).

The results summarised in Fig. 1 demonstrate that the levels of expression of RNA and p30 of either RD-114 or FeLV generally segregate into two distinct groups, one representing normal and the other lymphoma tissues. The majority of values for the normal tissues cluster below 10 ng per mg cell RNA or protein. In contrast the levels of both RNA and p30 of the endogenous xenotropic virus, RD-114, were considerably higher in the lymphoma tissues. In general, a good correlation was observed between the presence of RD-114-specific RNA and p30 in lymphoma tissues, with the exception of a few tissues which exhibited low but detectable levels of RNA expression in the absence of p30. This finding suggests that, although transcription of the RD-114 genome may occur in certain feline lymphomas, the entire genome is not necessarily translated. Many but not all of the lymphomas also exhibited elevated levels of FeLV RNA and p30 as compared with the normal tissues. Interestingly, most of the FeLV p30-negative lymphomas originated from older cats. As shown in Fig. 2, in which animals ⩽ 5 yr of age have been classified as younger cats and those ⩽ 7 yr as older cats, a general correlation was observed between the age of cats and virus gene expressions in their lymphomas. Whereas RD-114 RNA and p30 were expressed in the lymphomas of both younger and older animals, FeLV expression seemed to be most prevalent among cats ⩽ 5 yr of age. All but two lymphomas from cats of this younger group contained FeLV RNA (⩾ 25 ng mg^{-1}) and high p30 levels (⩾ 180 ng mg^{-1}) Moreover, there was an excellent correlation between elevated p30 levels and systemic infection with FeLV in these younger cats[16]. Thus, the detection of FeLV p30 and RNA expression in these animals probably reflected the presence of horizontally transmitted infectious FeLV. The vast majority of the lymphomas from the older cats, on the other hand, did not contain detectable FeLV p30, although some exhibited levels of RNA significantly higher than those found in normal tissues. The presence of RD-114 gene products and low or no expression of FeLV genes were consistently observed in multiple lymphoma tissues of individual old cats (data not shown).

The geometric mean values of the data and the statistical significance of the observed differences between the normal and lymphoma tissues from old and young cats are summarised in

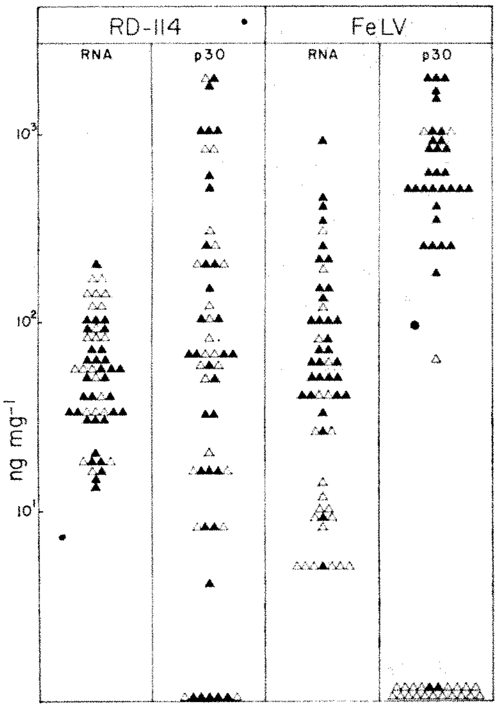
Table 1 Virus-specific RNA and antigen (p30) expression in normal and lymphoma tissue of cats of different age groups

		Young cats (≤ 5 yr)			Old cats (≥ 7 yr)		
		Normal	Lymphoma		Normal	Lymphoma	
Virus	Assay	(35)	(32)	*P*	(24)	(22)	*P*
RD-114	RNA	7.1	46.1	<0.0005	8.4	65.8	<0.0005
	p30	1.6	48.3	<0.0005	1.3	59.0	<0.0005
FeLV	RNA	5.8	81.7	<0.0005	7.8	17.1	<0.0100
	p30	1.7	414.0	<0.0005	2.4	2.3	<0.5000

Viral RNA and p30 concentrations were determined as summarised in Fig. 1. Values within parentheses represent the number of tissues tested. The geometric mean values listed represent ng virus-specific RNA or p30 per mg cell RNA or protein. *P* values were calculated using paired Student's *t* test of the logarithmic values of the ng mg^{-1} data.

Table 1. The differences in the expression of RD-114 genes in the lymphoma and normal tissues, at RNA or p30 level, are highly significant ($P < 0.0005$) irrespective of the age of the cats. Equally significant is the difference in the FeLV gene expressions in the lymphomas of young cats and the normal tissues from the corresponding age group. In contrast, no significant difference was found in the FeLV expression at the p30 level between the lymphomas from old cats and the corresponding normal tissues. The data suggest, however, a significant transcription of FeLV RNA in the absence of detectable p30 in a few lymphomas of old cats. Since sequences related to FeLV in the absence of infectious FeLV can be detected in the genomes of domestic cats[9,17,18], this transcription could represent either expression of these endogenous FeLV sequences, or subinfectious expression of exogenous FeLV acquired, perhaps, at an earlier age. Thus, it seems that the expression of the endogenous xenotropic RD-114 virus information is closely associated with lymphomas in cats of all ages, whereas FeLV related gene expression is primarily associated with lymphomas of younger cats. The absence of FeLV gene expression in many older and a few younger lymphomatous cats confirms our earlier findings based on other assays for FeLV (ref. 19), and suggests that, in these particular cats, spontaneous lymphoma may not be caused by FeLV. The widespread occurrence of RD-114 viral gene products in lymphomas suggests that expression of certain functions of this endogenous virus may be involved etiologically in the development of spontaneous lymphoid tumours of the cat. The alternative possibility, however, that elevated endogenous viral gene expression may be the result, rather than the cause, of neoplastic transformation cannot be ruled out by the present findings. It is our hope that elevated endogenous viral gene expression, even if not causatively involved in lymphomagenesis, may provide a logical approach towards immunoprevention.

Fig. 2 Virus-specific RNA and p30 expressions in lymphomas from young and old cats. Values were calculated as in Fig. 1. Cats ≤ 5 yr of age were designated as young and ≥ 7 yr of age as old. There were no 6-yr-old cats in this study. ▲, Young; △, old cats.



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Persistence of virus-specific memory B cells in mice CNS

IMMUNOGLOBULIN (Ig) populations detected in the cerebrospinal fluid (CSF) in chronic viral infections of the central nervous system (CNS) differ from serum Ig with regard to the relative concentration of antiviral antibodies[1-4], their light chain ratio[5] and their heterogeneity[6,7]. Such observations support the idea that antibodies are produced locally in the CNS[8]. But differences between CSF and serum Ig can also be explained in terms of the selective retention and accumulation of serum antibodies after passage across the blood–brain barrier[9]. Unequivocal proof of the local antibody production requires, however, that plasma cells, or their precursor cells, be isolated from the CNS. Intracerebral inoculation of mice with 6/94, a para-influenza type 1 virus[10], induces an immunologically mediated degeneration of the white matter which is related to infiltration of the brain parenchyma by mononuclear cells[11,12]. In the study reported here, cells recovered from the brains of virus-infected mice were investigated as described previously to analyse memory B cells reactive to influenza viruses[13,14]. Virus-reactive memory B cells, that is immunocompetent cells able to generate, on antigenic stimulation, a clone of antiviral-antibody-producing plasma cells, were demonstrated in the brains of mice infected intracerebrally with 6/94 virus but not in the brains of mice injected intraperitoneally.

BALB/c mice were inoculated intracerebrally or intraperitoneally with 6/94 virus. At various times the 6/94-reactive memory B cells obtained from brain, blood and spleen of individual donor mice were counted in an adoptive transfer system described previously[13]. Cells used for adoptive transfer were prepared as follows. Blood was withdrawn by heart puncture and red cells were lysed by incubation in NH_4Cl (ref. 15). White cells were pelleted through a cushion of calf serum[15] and resuspended in a balanced salt solution (BSS). A spleen cell suspension was prepared by gentle disruption of the spleen in a tissue homogeniser[13]. To obtain brain cells the brain was removed aseptically, rinsed in cold BSS, finely diced with a tissue chopper (McIlvain, Brinkman) and resuspended in ice-cold BSS. Large brain fragments were allowed to settle and the supernatant was withdrawn and spun for 8 min at 800*g* at 4 °C. The pellet was resuspended in BSS and mixed with a solution of bovine serum albumin (BSA) in BSS to give a final BSA concentration of 21%. A discontinuous BSA gradient was prepared, as described by Shortman[15], by underlaying a 32% BSA solution and overlaying BSS. The gradient was spun for 20 min at 3,000*g*. Cells that had accumulated at the interface between 32% and 21% BSA were collected, spun out of the BSA and resuspended in BSS. Between 1 and 4×10^5 viable cells (Trypan blue exclusion) were recovered from a single brain. The BSA purification of the brain extract was necessary because the intravenous injection of crude brain eluate produced a high death rate in recipient mice, probably caused by microemboli. Each cell preparation was then injected intravenously into lethally irradiated syngeneic recipient mice[13]. Splenic fragments of the recipient mice were cultured individually (50–70 fragments per spleen) and analysed by radioimmunoassay (RIA)[14] for the production of antibodies to 6/94 virus after secondary stimulation *in vitro* with 6/94. In these conditions, the number of antibody-producing splenic fragments has been shown to be correlated with the number of transferred precursor B cells[13].

The results of several adoptive transfer experiments with cells from the brain of mice at various times after intracerebral or intraperitoneal inoculation with 6/94 are shown in Tables 1 and 2, respectively. Two points are noteworthy. First, 6/94-reactive memory B cells could be demonstrated in the brain cell eluate from all but two of the intracerebrally inoculated mice. Although the absolute numbers of detected foci per experiment was small (because of the paucity of cells recovered from brain) the relative frequency (per 10^5 transferred cells) of 6/94-reactive memory B cells was, in the positive experiments, of the same magnitude or higher than in circulation or spleen. Second, virus-reactive memory B cells seemed to persist in the brains of individual mice for at least 4–7 months after intracerebral inoculation.

Table 1 6/94-reactive memory B cells from mice inoculated intracerebrally with 6/94 virus

Weeks after i.c. infection of donor mice*	No. of anti-6/94 antibody-producing splenic fragments per 10^5 transferred cells		
	Brain	Circulation	Spleen
4	0.9 (2/2.2)	0.07	0.41
5	10.0 (5/0.5)	0.25	1.0†
7	0.8 (2/2.5)	0.73	0.60
9	< 0.2 (0/4.8)	0.82	2.0†
18	0.6 (3/4.8)	0.70	0.80
31	(10/half brain)	ND	ND
34	(None/half brain)	< 0.2	1.10

To establish that no background antibody production occurred with any given batch of recipient mice, each experiment included fragment cultures from lethally irradiated mice that had not received adoptive transfer of cells. All these control cultures in the experiments included in Tables 1 and 2 were negative.

* BALB/c mice were inoculated intracerebrally (i.c.) with approximately $10^{6.3}$ EID_{50} of 6/94 and were killed at the times indicated and cells prepared for use in the adoptive transfer. Antiviral-antibody-producing splenic fragments were counted 12 and 15 d after their secondary stimulation *in vitro* with 6/94.

† There were too many transferred cells for an accurate determination of precursor cell frequency. ND, not determined. Figures in parentheses show total numbers of foci observed per total number of transferred cells × 10^5.

Our results (excluding the final two lines of Table 1), can be summarised to show that antiviral antibody was produced by 12 splenic foci obtained after transfer of 14.8×10^5 brain eluate cells after intracerebral inoculation; conversely, no antibody was produced by any of the spleen fragments after transfer of 9.7×10^5 brain eluate cells after intraperitoneal inoculation. It is thus evident that 6/94 memory B cells were detected in the brain eluate only after intracerebral inoculation of donor mice. Given that the number of 6/94-reactive memory B cells in the circulation was approximately the same after inoculation by both routes (Tables 1 and 2), and assuming that the degree of contamination of the brain eluate cells by circulating blood cells was the same in both intracerebrally and intraperitoneally inoculated mice, we conclude that the 6/94 memory B cells in the brain eluate cell preparation of intracerebrally inoculated mice were previously localised in the brain parenchyma and did not represent contaminating blood cells. This is supported by the results of experiments done 4 and 5 weeks after intracerebral inoculation when, on adoptive transfer, the brain eluate cells produced 10–40 times (Table 1) more splenic foci than did the same number of white blood cells of the given donor mice.

In the analysis reported here, the comparison was between the number of 6/94-reactive precursor B cells and the total number of

Table 2 6/94-reactive memory B cells obtained from mice inoculated intraperitoneally with 6/94 virus

Weeks after i.p. infection of donor mice*	No. of antibody-producing splenic fragments per 10^5 transferred cells		
	Brain	Circulation	Spleen
3	< 0.4 (0/2.4)	0.27	ND
10	< 0.3 (0/2.9)	0.58	ND
18	< 0.3 (0/2.8)	0.44	1.25
34	< 0.6 (0/1.6)	0.20	0.92

See legend to Table 1.

* BALB/c mice were inoculated intraperitoneally (i.p.) with approximately $10^{7.3}$ EID_{50} of 6/94. ND, not determined.

transferred cells, regardless of type. It would be more useful to correlate the number of 6/94-reactive precursor B cells with the number of transferred B cells. It is not known what proportion of the cells eluted from the brain consists of B cells, but it is presumed to be smaller than the proportion of B cells in white blood cells (approximately 20%, refs 16 and 17) or spleen cells (approximately 40%, ref. 18). Thus our data probably underestimate the actual difference in terms of absolute frequency (per transferred B cell) of 6/94-reactive memory B cells in the brain of intracerebrally infected mice as opposed to the circulation or the spleen.

This study provides the first direct evidence for the presence of virus-reactive memory B cells in brain tissue of mice with a virus-induced neurological disease. A local immune response can thus be maintained within the CNS.

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H–2 gene expression is required for T cell-mediated lysis of virus-infected target cells

EFFECTOR T cells generated during the course of virus diseases of mice interact only with virus-infected target cells sharing H–2K or H–2D antigenic specificities with the mouse strain in which the lymphocytes are sensitised[1–3]. The current interpretation[4] of this phenomenon is that the cell membrane component(s) recognised by the T-cell receptor(s) has characteristics of both self (H–2) and non-self (virus). Phenotypic expression of H–2 genes in the target cell would thus be essential for T cell-mediated immunity. Here we test this hypothesis using two teratocarcinoma cell lines (F–9 and PCC4 derived from strain 129 mice[5,6]) infected with lymphocytic choriomeningitis virus (LCMV). We show that the F–9 tumour cells apparently do not express H–2K or H–2D antigenic specificities (Fig. 1, Table 1), and are also resistant to attack by lymphocytes sensitised against LCMV. The PCC4 line, however, has remained multipotential and can give rise to various different cell types, at least some of which show evidence of H–2 gene function (Table 1) and are attacked by the appropriately sensitised lymphocytes.

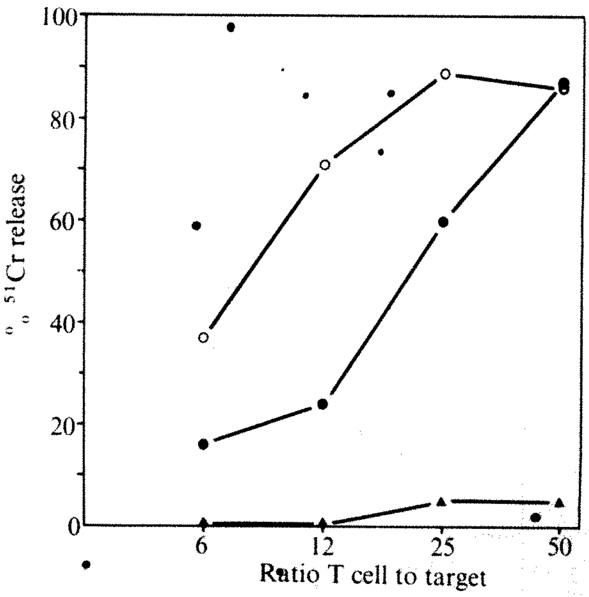

Fig 1 The F–9 teratocarcinoma cell is not lysed by alloreactive T cells specific for H–2^b antigens, expressed on MC57G tumour cells and C57BL primary peritoneal macrophages. Cytotoxic effectors were generated by secondary stimulation of memory lymphocytes from BALB/c mice with mitomycin C-treated (50 μg ml^{-1}) C57BL spleen cells[15]. The assay was incubated for 16 h at 37 °C and results are expressed as % specific ^{51}Cr release.

Equivalent amounts of cell-surface viral antigen are found on immunofluorescent examination[7] of unfixed, LCMV-infected F–9, PCC4 and MC57G (H–2 positive) targets. Maximal virus-specific T-cell effector function is, however, recognised only for the MC57G cell line (Fig. 2). Apparently some PCC4 cells are lysed, but LCMV-infected F–9 cells are evidently not damaged by virus-immune lymphocytes (Fig. 2). Repeated experiments with the PCC4 targets gave extremely variable results (from 21% to 90% of the virus-specific ^{51}Cr release found for MC57G) and, although the two series of observations have not yet been made concurrently, the extent of H–2 antigen expression on PCC4 cells may also vary considerably (from 14% to 56%). The limiting factor in virus-specific T cell-mediated immunity would thus seem to be H–2 gene expression in the target cell. This is in accord with the observation that blocking of tumour-specific effector T cells may be achieved by treating the targets (but not the lymphocytes) with anti-H–2 serum[8,9], and with previous experiments using trinitrophenyl (TNP)-modified F–9 cells[10].

Exposure of the cell membrane to TNP might be thought to induce direct alteration of the H–2 antigen, which would obviously not occur if the antigen is absent. However, infectious virus which

Table 1 Presence of serologically-detectable H–2 antigens

Target cells	Mouse strain and H–2 type	% Positive by immunofluorescence			
		anti-H–2K^b (33, 53, 54)	anti-H–2D^b (2, 28, 59, 56)	anti-H–2D^b (2, 56)	Normal C57BL
F–9	129, H–2^{bc}	< 1	< 1	5	4
PCC4	129, H–2^{bc}	42	16	30	2
MC57G	C57BL, H–2^b	98	ND	91	4
Spleen	129, H–2^{bc}	96	99	ND	32*
Spleen	C3H, H–2^k	27*	32*	ND	33*

Mouse alloantisera 33, 28b and D–2, reactive to the H–2 specificities shown above in parentheses, were supplied by the Transplantation and Immunology Branch, National Institute of Allergy and Infectious Diseases, NIH. Fluorescent rabbit IgG specific for mouse IgG heavy and light chains were purchased from Cappel Laboratories, Downington, Pennsylvania. All sera were used at a dilution of 1:10.

* Reactivity is due to Ig-bearing B cells and antibody-forming cells in the spleen population.

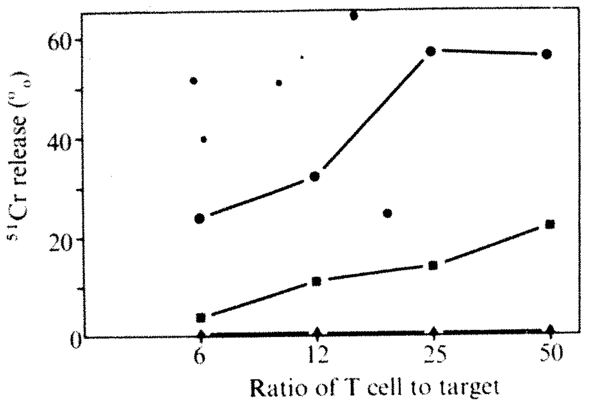

Fig. 2 The extent of specific lysis of virus-infected target cells by virus-immune effector T cells from C57BL mice injected with 1,000 LD_{50} of WE3 LCMV 8 d previously. The assays[16] were incubated for 16 h at 37 °C.

has been introduced to the cell at a relatively low multiplicity (5 LD_{50} per cell 48 h before assay), could potentially modify cell-surface structure in other ways. Perhaps the process of virus growth causes derepression of cryptic genes within the H-2 complex, and the "virus-immune" T-cell response is directed solely against abnormally expressed H-2 antigen[11]. Alternatively some "interaction antigen"[12,13] may be formed, which is partly coded for by H-2 genes and partly by the viral genome. Apparently neither of these mechanisms, which could conceivably be independent of phenotypic expression of normal H-2 antigen, operates in the virus-infected F-9 cell or is obviously relevant to the variability observed with PCC4. Perhaps the necessary genes have been deleted from the F-9 tumour, although these cells express a closely-linked gene product which may be the embryonic equivalent of H-2 (ref. 14).

Use of this experimental system also establishes that absence of H-2K or H-2D cell-surface antigens in no way facilitates lysis mediated by virus-immune T cells (differing in H-2 specificity) (Table 2). It seems likely therefore that the presence of foreign H-2K or H-2D determinants does not inhibit lymphocyte function. Occurence of virus-specific cell-mediated immunity apparently depends on recognition by the lymphocyte of some cell membrane component coded for by the H-2 gene complex. The possibility that H-2K and H-2D antigens function as "self-markers" in cell-mediated immunity[4] must be considered.

Table 2 Absence of H-2 does not facilitate lysis of different H-2 specificity by virus-immune T cells

Immune spleen	% ^{51}Cr release from virus-infected targets		
	MC57G	F-9	B10.A
B10 ($H-2^b$)	39	0	5
B10.A ($H-2^a$)	0	1	78
CBA/J ($H-2^k$)	0	0	25

Mice were injected with 1,000 LD_{50} of WE3 LCMV 8 d previously, and spleen cells and targets were mixed in a ratio of 30:1 (ref. 16). The B10.A and CBA/J mouse strains share $H-2K^k$ antigenic specificities. The B10.A target cells were peritoneal macrophages.

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A recombinant in the major histocompatibility complex of the rat

WE report here a recombinant in the major histocompatibility complex (MHC) of the rat, and describe the properties of alloantisera directed at the recombined portions of one haplotype. The results are compatible with a structure for the rat MHC more closely resembling that of man than that of the mouse.

The inbred rat strains used were HO(PVG/c) (AgB5 = $H-1^c$), DA(AgB4 = $H-1^a$) and AO (AgB2 = $H-1^u$). An historical accident has led to the use of two parallel nomenclatures for the rat MHC. We use the AgB terminology of Palm[1] without prejudice to a future reconciliation with the Rat H-1 terminology of Stark *et al.*[2]

The recombinant was discovered among progeny of the backcross HO ♂ × (DA × HO)F_1 ♀. The progeny were screened with three tests defining the segregating AgB4 allele:

(1) For the presence or absence of AgB4 alloantigens on the surface of their blood lymphocytes recognised by the alloantiserum (AO × HO)F_1 anti-DA in a one-stage cytotoxic test assayed by Trypan blue exclusion.
(2) For the ability or inability of their blood lymphocytes to stimulate HO thoracic duct lymphocytes (TDL) in a mixed lymphocyte interaction (MLI).
(3) For the ability or inability of their blood lymphocytes to respond in an MLI against (DA × HO)F_1 spleen cells.

The three tests gave highly concordant results on HO × (DA × HO)F_1 backcross progeny, indicating that all three tests defined a single genetic system (Table 1). One rat showed aberrant behaviour in that her cells, although killed by (AO × HO)F_1 anti-DA serum, responded to (DA × HO)F_1 spleen cells in MLI and did not noticeably stimulate HO TDL. This rat was backcrossed to the HO strain. Of her two litters, 8 out of 21 rats carried serological determinants recognised by (AO × HO)F_1 anti-DA serum, eliminating the slight possibility that the positive result in the serological test of the mother was caused by a rare co-segregation of several minor antigens recognised by the serum. The reactions in the three screening assays of one serologically positive member of these litters are shown in Table 1. The putative recombinant haplotype and tissue will provisionally be called 1R.

The next experiments showed that antigenic products of both the regions of AgB recombined in the 1R chromosome were serologically recognisable, but differed markedly in tissue distribution. First, (AO × HO)F_1 anti-DA serum was absorbed with 1R lymphoid tissue. DA lymphoid tissue absorbed all activity from the serum, whereas even a massive amount of 1R tissue absorbed the serum partly but not completely (Fig. 1*a*). As all the detectable activity of (AO × HO)F_1 anti-DA was directed against AgB4 (Table 1), the absorption result implies that the 1R haplotype carries some serologically detectable AgB4 alloantigens, but lacks others.

Suitable genetic combinations were therefore chosen to raise separate alloantisera against the two adjacent portions of AgB4. (1R × HO) heterozygotes were immunised against DA, and (AO × HO)F_1 rats against (1R × HO) heterozygous lymphoid tissue. In a lymphocytotoxic test the (AO × HO)F_1 anti-1R serum killed all lymphocytes in blood, lymph nodes and

Table 1 (AO × HO) anti-DA serum detects antigens of the MHC. Tests on a panel of HO × (DA × HO)F_1 backcross progeny

Rat no.	(AO × HO) anti-DA(1/192) % blood lymphocytes killed	Complement control % dead	MLI Response to (DA × HO)F_1 spleen	MLI stimulation of HO
1	4.1	4.4	4.32 (0.04) (A)	2.40 (0.23) (E)
2	8.1	6.3	4.10 (0.11) (A)	3.09 (0.16) (D)
3	8.7	9.8	4.30 (0.02) (A)	3.38 (0.20) (D)
4	97.1	4.4	2.67 (0.15) (A)	4.66 (0.03) (D)
6	97.6	7.2	2.62 (0.23) (B)	4.58 (0.09) (D)
25	94.9	7.4	2.58 (0.25) (B)	4.59 (0.03) (E)
27	96.9	5.9	2.68 (0.30) (B)	4.58 (0.06) (E)
31	96.3	5.0	2.91 (0.08) (C)	4.49 (0.21) (E)
32	4.3	4.0	4.44 (0.03) (C)	2.50 (0.07) (F)
34	96.0	4.6	2.76 (0.14) (C)	4.43 (0.06) (F)
38	94.3	4.2	2.97 (0.10) (C)	4.19 (0.08) (F)
1R heterozygote*	82.0	3.4	4.44 (0.05) (C)	2.21 (0.10) (F)

Experiment	Response control	Syngeneic control
A	4.25 (0.07)	2.75 (0.08)
B	4.39 (0.06)	2.48 (0.08)
C	4.11 (0.11)	3.05 (0.16)
D	4.69 (0.03)	3.38 (0.11)
E	4.65 (0.03)	3.22 (0.16)
F	4.22 (0.08)	2.17 (0.11)

Lymphocytotoxic tests were performed in a total volume of 75 μl, containing 5×10^4 target cells and guinea pig serum at a final concentration of 8–11% (v/v). The suspension medium was Dulbecco's A and B salts supplemented with 2% foetal calf serum. Percentage cell lysis was determined by Trypan blue exclusion, more than 270 cells being counted in each test. The complement control lacked any antiserum. Mixed lymphocyte cultures contained 2×10^5 responding cells and 3×10^5 stimulating spleen cells (irradiated with 950 rad of 16 MeV X rays), in RPMI medium supplemented with 5% rat serum and 2.5×10^{-5} M 2-mercaptoethanol in a total volume of 0.2 ml in round-bottomed microtitre plates. Methyltritiated thymidine (16 Ci mmol^{-1}; 0.1 μCi in 10 μl) was added at 90–100 h and cells were collected about 16 h later. For MLI responses to (DA × HO)F_1 spleen, responding lymphocyte populations were prepared from cardiac blood by Ficoll-Isopaque separation[3]. Spleen cells were used to stimulate HO thoracic duct lymphocytes. Mixed lymphocyte responses are expressed as the log$_{10}$ geometric mean c.p.m. of at least three replicate cultures. The standard deviation from the log$_{10}$ geometric mean c.p.m. in replicate cultures is in the first parentheses after the response. In the second parentheses, A–F are names for the experiments for which the response controls and syngeneic controls are given below the main body of the table; in A–C the response control is the response of HO blood lymphocytes to (DA × HO)F_1 spleen; in D–F the response control is the response of HO thoracic duct lymphocytes to (DA × HO)F_1 spleen.

*The results for 1R heterozygote were obtained with one of the original recombinant's second litter by an HO male.

thoracic duct of rats carrying AgB4 (Fig. 1*b*). It was also strongly and specifically lytic for red cells of such rats. The target antigen(s) on both red cells and lymphocytes is AgB-linked as established through five backcross generations of both AgB4 and the 1R haplotype on to the HO background. The target(s) for this antiserum seems therefore to be analogous to the mouse H-2K and H-2D molecules, which are also expressed on both lymphocytes and erythrocytes[4,5].

The (1R × HO) anti-DA serum was considerably different. Although of high titre, it killed only 30–40% of normal lymphocytes (Fig. 1*b*), and was not detectably haemolytic. Furthermore, it seemed that the target for this antiserum was restricted to B lymphocytes. Using lymphocytes collected from the thoracic duct of a thymectomised, irradiated, bone-marrow reconstituted 'B' rat[6] of the DA strain it was found that > 90% of 'B' TDL were killed by (1R × HO) anti-DA serum while only 35–40% of normal TDL were killed in the same test (Fig. 1*b*). Similarly, the (AO × HO)F_1 anti-DA serum absorbed exhaustively with 1R lymphoid tissue killed about 90% of 'B' TDL compared with 35–40% of normal TDL (Fig. 1*a*). (1R × HO) anti-DA serum is thus analogous to some mouse anti-Ia sera in the apparent absence of the target antigens from erythrocytes and from T cells[7,8]. It was necessary to demonstrate that the targets of (1R × HO) anti-DA and (AO × HO)F_1 anti-1R are controlled by linked portions of AgB. For this purpose we returned to the progeny of the HO × (DA × HO)F_1 backcross amongst which the putative recombinant was found. The two antisera were tested on blood lymphocytes of a panel of these rats whose AgB status had already been established. There was complete positive correlation of the activity of the two antisera and both detected antigens of AgB4 (Table 2). Thus (1R × HO) anti-DA and (AO × HO)F_1 anti-1R are directed at antigens controlled by different segments of a single genetic system.

The recombinant haplotype, 1R, has inherited its major serological antigens (analogous to the mouse K and D molecules) from the AgB4 of DA, and lacks antigen(s) of AgB4 seemingly analogous to mouse Ia antigens. It has also inherited its MLI phenotype principally from AgB5, the (1R × HO)

Table 2 Genetic linkage test for the targets of (AO × HO)F_1 anti-1R and (1R × HO) anti-DA using a panel of HO × (DA × HO)F_1 backcross progeny

Rat	AgB4	Complement control (% cells dead)	% cells killed by (1R × HO) anti-DA		% cells killed by (AO × HO) anti-1R	
			1/6	1/24	1/6	1/192
(DA × HO)F_1	+	6.1	ND	23.0	ND	92.1
26	+	7.8	ND	27.7	ND	96.9
30	+	4.2	ND	28.2	ND	94.6
42	+	8.0	ND	23.0	ND	94.4
44	+	11.4	ND	25.2	ND	93.5
45	+	5.5	ND	20.6	ND	93.6
46	+	4.4	ND	20.6	ND	95.3
33	—	7.5	7.3	6.7	5.6	4.9
35	—	11.8	9.9	7.1	8.6	11.1
36	—	3.7	3.5	2.3	3.2	5.1
37	—	7.3	5.5	7.5	6.5	6.9
43	—	5.0	6.9	2.5	5.5	6.6

The AgB status of each of these animals was determined by both serological and MLI tests, as described in Table 1. Lymphocytotoxic scores are based on a sample of > 300 cells. ND, not determined.

Table 3 MLI responses of HO and (1R × HO) heterozygote TDL to (DA × HO)F_1 spleen

Responder TDL (2×10^5)	Stimulator spleen cells (3×10^5)	Response
HO	HO (syngeneic control)	2.58 (0.02)
	(1R × HO) heterozygote*	2.47 (0.08)
	(DA × HO)F_1	4.73 (0.03)
(1R × HO) heterozygote*	HO	2.66 (0.03)
	(1R × HO) heterozygote (syngeneic control)	2.46 (0.05)
	(DA × HO)F_1	4.72 (0.04)

MLI Response data is presented as in Table 1.
*The (1R × HO) heterozygotes used in this experiment were offspring of the original recombinant, previously shown to be carrying DA serological determinants by positive reaction with (AO × HO)F_1 anti-DA serum.

heterozygote behaving just like HO in this experiment (Tables 1 and 3). Five backcrosses of the 1R haplotype on to the HO background have now been completed.

The analysis of this recombinant demonstrates that the region of AgB controlling the MLI and Ia-like antigens can be recombined by a single cross over from the region controlling cell-surface alloantigens of wide tissue distribution analogous to the K and D molecules of the mouse. The properties of the recombinants described by Williams and Moore[9], of the KGH rat strain investigated by Gill and co-workers[10,11] and of the AgB haplotype derived from a wild rat by Gasser[12] support this view. Although the existence of more than one K/D-like locus has not been demonstrated in the rat, it seems that the linear order of genes in AgB is not like that in the H-2 of the mouse, where the D and K loci flank the region controlling the MLI and Ia antigens. The rat may fit better the model established for the human MHC[12]. Indeed, the present study further emphasises the conclusion derived from other more distant mammalian species including man[13], that the gene order in the mouse MHC is exceptional, presumably as a result of a chromosome rearrangement which can now be placed as more recent than the divergence of the mouse and the rat from their common ancestor. We consider, however, that our conclusion on the structure of the rat MHC is still preliminary. Confirmation from other more informative laboratory recombinants is awaited, because special circumstances such as the undetected sharing of a second K/D-like chromosome region by the AgB4 and AgB5 haplotypes could invalidate the conclusion.

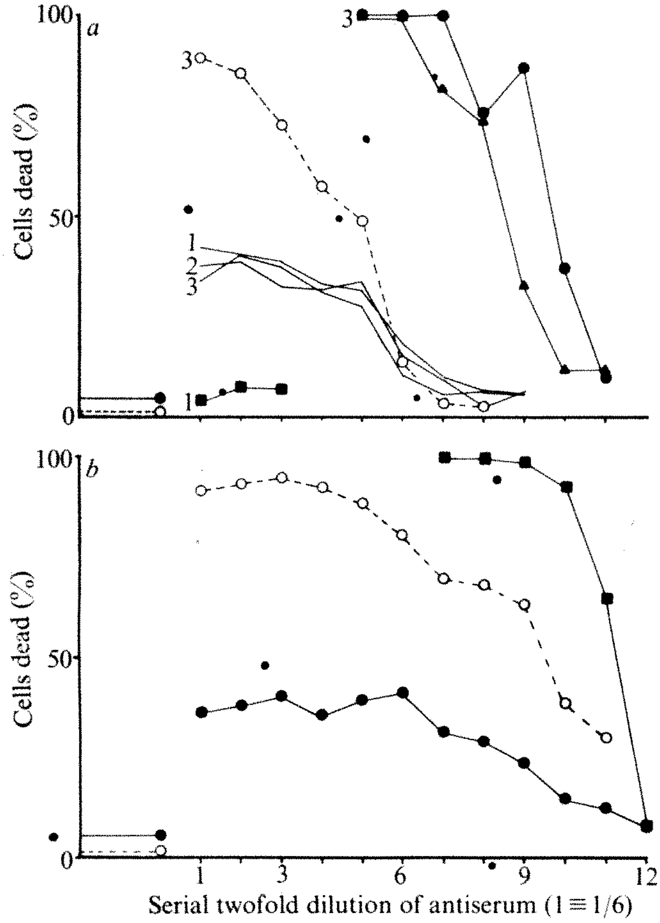

Fig. 1 Titrations of cytotoxic antisera against DA lymphoid cells (methods as in Table 1). *a*, (AO × HO)F_1 anti-DA absorbed with 1R lymphoid tissue. (●———●), (AO × HO) anti-DA tested on DA lymph node cells: (———), (AO × HO) anti-DA absorbed with 1R tested on DA lymph node cells. 1, 2, 3 are successive stages in the absorption. At each stage more than 700×10^6 lymphocytes were used per ml of antiserum; (○— — —○), (AO × HO) anti-DA absorbed with 1R at stage 3 of absorption, tested on 'B' DA TDL; (■———■), (AO × HO) anti-DA absorbed with DA at stage 1; (▲———▲), control absorption with HO at stage 3; (———●), complement control for DA lymph node cells; (— — —○) complement control for 'B' DA TDL. *b*, Recombinant antisera tested on DA TDL. (■———■), (AO × HO)F_1 anti-1R on normal DA TDL; (●———●), (1R × HO) anti-DA on normal DA TDL; (○———○), (1R × HO) anti-DA on 'B' DA TDL. Complement controls as in (*a*).

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Note added in proof: A recombination between AgB4 and AgB2 (H−1^a and H−1^w) has recently been found[15]. This recombinant seems to have similar properties to that we have described.

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A surface antigenic marker for rat Schwann cells

THE use of cell-surface antigenic markers to identify and separate distinct subpopulations of lymphocytes has revolutionised immunology in recent years. It seems likely that a similar approach will be fruitful in other areas of biology. For example, recent advances in maintaining and studying cells of the nervous system *in vitro* have created a pressing need for cell-type-specific markers. These would allow unequivocal identification and possible purification of the many different types of neural cells so that their interactions can be studied in culture. Here we describe a cell-surface antigen, defined by an antiserum raised in mice against a rat neural tumour cell line[1] which is present on Schwann cells in dissociated cell culture of neonatal rat sciatic nerve, but not on fibroblasts in the same culture. Another cell-surface antigen, Thy-1.1 (formerly called θAKR)[2] is expressed by the fibroblasts[3] and not the Schwann cells, making it possible to mark both cell types simultaneously in these cultures by using antibodies conjugated to two different fluorochromes.

The mouse antiserum was raised against a cell line (33B) derived from an ethylnitrosourea-induced tumour arising in the spinal cord and nerve roots of a Wistar–Furth (W/Fu) rat[1]. After extensive adsorption with rat liver, spleen and thymus, the original serum defined two surface antigens detected by cytotoxicity testing: (1) a 'common antigen' present in rat brain, embryonic tissues and most tumours or cell lines from the rat nervous system (including Schwannomas, gliomas and neuroblastomas) and (2) a 'restricted antigen' present on a minority of nervous system tumours and cell lines and not detectable in normal or embryonic rat tissues[1]. For the studies described here, we used an antiserum, raised in A/strong mice[4], with cytotoxic activity at least 80% specific for the common antigen. After the usual tissue adsorptions, it was further adsorbed twice with half its volume of packed cells obtained from primary muscle cultures of neonatal W/Fu rats. The antigen detected by immunofluorescence on Schwann cells has a similar distribution on tumour cell lines to that of the common antigen (see below). The exact identity of the Schwann cell antigen and the common antigen is, however, not yet completely established. We shall therefore refer to the antigen detected on Schwann cells as rat neural antigen-1 (Ran-1).

Table 1 Indirect immunofluorescence labelling of Schwann cells and fibroblasts in rat sciatic nerve cultures with anti-Ran-1 and anti-Thy-1 antiserum

Antiserum	Adsorbed with	Labelling of Schwann cells	Labelling of Fibroblasts
Mouse anti-Thy-1.1	—	0	+
Mouse anti-Thy-1.1	CBA (Thy-1.2) brain	0	+
Mouse anti-Thy-1.1	AKR (Thy-1.1) brain	0	0
Rabbit anti-Thy-1	—	0	+
Rabbit anti-Thy-1	Rat (W/Fu) thymocytes	0	0
Mouse anti-Ran-1	—	+	0
Mouse anti-Ran-1	Rat neural tumour line 33B	0	0
Mouse anti-Ran-1	Rat neural tumour line 21A	0	0
Mouse anti-Ran-1	Rat neural tumour line B28	+	0
Mouse anti-Ran-1	Rat foetus	0	0
Mouse anti-Ran-1	Rat liver	+	0

Cells were labelled with mouse anti-Thy-1.1 (1:5) or mouse anti-Ran-1 (1:50) and then G anti-MIg-Rd (1.20), or with rabbit anti-Thy-1 (1:20) and mouse anti-Ran-1 (1:50) simultaneously, followed by F(ab)$_2$ G anti-RIg-Fl (1:10) and G anti-MIg-Rd (1:20) applied simultaneously.

Anti-Thy-1.1 alloantiserum was raised in CBA mice against AKR thymocytes as described before[5]; in indirect immunofluorescence assays it labelled AKR and rat thymocytes but not CBA thymocytes, and all of this activity could be adsorbed by brain from AKR but not from CBA mice. Rabbit anti-Thy-1 antiserum was raised by immunising rabbits with rat brain glycoproteins (obtained after lentil lectin affinity chromatography) and boosting with purified Thy-1 5 months later[6]. It was adsorbed twice with 50% (v/v) packed rat spleen cells and twice with 50% (v/v) packed neonatal rat brain homogenate (both of which are known[7,8] to contain relatively little Thy-1). For indirect immunofluorescence assays, rhodamine-conjugated goat anti-mouse IgG (G anti-MIg-Rd) and fluorescein-conjugated goat anti-rabbit IgG (G anti-RIg-Fl) antibodies Nordic batch no. 2–773 and 27–175, respectively) were used. In most experiments, the F(ab)$_2$ fragments of these conjugates were used. They were prepared by dialysing the undigested reagents in 0.1 M acetate *p*H 4.5 and incubating them with 2% (w/w) pepsin (Sigma) for 12 h at 37 C, dialysing in phosphate buffered saline and passing them through an XM50 Amicon filter to remove the Fc fragments. All antisera were centrifuged at 100,000*g* for 30 min before use, and for double labelling experiments the G-anti-MIg-Rd was adsorbed with rabbit IgG and the G anti-RIg-Fl with mouse IgG. The rabbit and mouse IgG were coupled to Sepharose 6B.

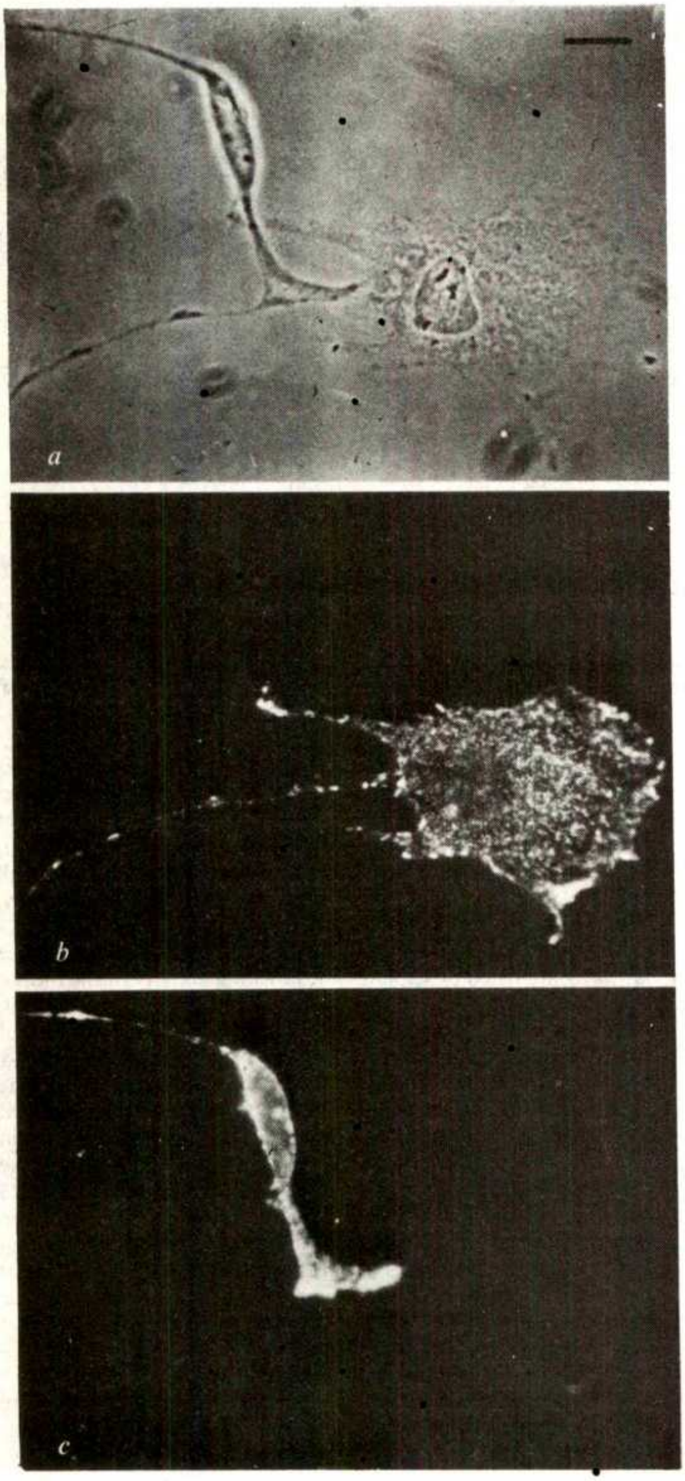
Sciatic nerves were removed aseptically from neonatal W/Fu rats, digested with trypsin and collagenase, and dissociated by passage through a No. 23 hypodermic needle. The cells were grown on collagen-coated glass coverslips in Linbro dishes (Flow Labs.) in Dulbecco's modified Eagle's medium, containing penicillin (100 IU per ml), streptomycin (100 μg ml^{-1}), 10% heat inactivated foetal calf serum and 2% chick embryo extract. A full account of this procedure will be published elsewhere. After 3–5 d of culture, the cells on the coverslips were incubated in anti-Ran-1 or anti-Thy-1 antisera for 30 min at room temperature, washed well and incubated with the appropriate fluorescent anti-Ig antibodies for a further 30 min at room temperature. After washing, the cells were fixed in 5% glacial acetic acid in 95% ethanol at 4 °C for 10 min, mounted in Elvanol on a glass slide and examined using a Zeiss fluorescence microscope equipped with phase contrast and incidence fluorescence optics.

In 3-d-old cultures, the majority of cells had a bipolar morphology characteristic of Schwann cells in culture[9] with a tendency to line up end-to-end; by 5 d the long processes of these cells tended to form interconnected networks. The other cells in the culture had the appearance of fibroblasts; initially sparse, they tended to proliferate and form a confluent underlayer by 7 d. Every one of the characteristic bipolar Schwann cells was brightly labelled by the anti-Ran-1 serum and did not label with the mouse or rabbit anti-Thy-1 antiserum. On the other hand, all of the typical fibroblasts were labelled by both of the anti-Thy-1 sera, and not by the anti-Ran-1 serum. In all cultures, there were cells with an intermediate morphology, which stained as typical Schwann cells.

The controls for the specificity of the immunofluorescence labelling are listed in Table 1. The labelling activity of the mouse anti-Thy-1.1 antiserum was removed by adsorption with AKR but not CBA brain, confirming the Thy-1.1 specificity of the antiserum. The labelling activity of the rabbit anti-Thy-1 serum was removed by adsorption with rat thymocytes. The labelling of Schwann cells by anti-Ran-1 could be removed by adsorption with cell line 33B, which has both the common and the restricted antigen, or by line 21A, which has the common antigen but lacks the restricted antigen. Line B28 (derived by D. Schubert at the Salk Institute from a brain tumour) lacks both antigens and does not adsorb the Schwann cell labelling. Adsorptions with rat brain, which contains less of the antigen than the tumour lines or rat foetus, have thus far been inconclusive. With this reservation, these data suggest that the immunofluorescent labelling of Schwann cells is detecting the common antigen.

To prove that the anti-Ran-1 and anti-Thy-1 antisera were not labelling the same cells, cultures were exposed to rabbit anti-Thy-1 and mouse anti-Ran-1 antisera simultaneously, washed and then incubated in G-anti-RIg-Fl and G anti-MIg-Rd simultaneously; after washing, the cells were examined for fluorescein and rhodamine fluorescence. In most cultures, all cells labelled with one or other fluorochrome but not both (Fig. 1; also see cover). In an occasional culture, a small proportion (< 5%) of the cells were labelled with both fluorochromes; these cells had a distinctive round morphology and were also doubly labelled when normal mouse and rabbit sera were used in the first incubation in place of the antisera. They could be damaged cells or macrophages.

Fig. 1 Indirect immunofluorescence using antibodies against Thy-1 and Ran-1. Cells from sciatic nerve cultures were stained with a mixture of rabbit anti-Thy-1 and mouse anti-Ran-1, followed by a second incubation with $(Fab)_2$ G-anti-RIg-fluorescein and $F(ab)_2$ G anti-MIg-rhodamine, as described in the text. A pair of cells was photographed with phase optics (a) or ultraviolet illumination and filters to detect the fluorescein (anti-Thy-1)-labelled fibroblast (*b*), or filters to detect the rhodamine (anti-Ran-1)-labelled Schwann cell (*c*) Scale bar, 10 μm.



In preliminary experiments, the double labelling method has been used to investigate cell division in the two populations. Two-day cultures were incubated with ^{3}H-thymidine for 24 h, stained with the two fluorochromes, and coated with emulsion for conventional light microscope autoradiography. Whereas approximately 90% of the Thy-1 positive cells had labelled nuclei, only 20% of the Ran-1 positive cells had them. The latter result is comparable with that obtained in neonatal mouse sciatic nerve *in vivo*[10]. This method should be useful in the search for possible Schwann cell mitogens.

We conclude from these studies that in dissociated cell cultures of newborn rat sciatic nerve it is possible to distinguish Schwann cells from fibroblasts by means of surface antigenic markers: the Schwann cells express the previously described[1] Ran-1 but not detectable amounts of Thy-1, while fibroblasts do not express detectable amounts of Ran-1 but do express Thy-1. Although the Thy-1 positive cells had a typical fibroblastic morphology, it is possible that this category may include other cell types. Thus, endothelial cells are present in peripheral nerve[10,11] and they have not been typed for the presence of Thy-1. The identity of the Ran-1 positive cells with intermediate morphology is not seriously in doubt. Studies in culture with time-lapse cinematography[12] have shown that bipolar Schwann cells may undergo a morphological transition to a fibroblast-like form. Although the detailed distribution of Ran-1 in the nervous system is not known, it is not detectable by immunofluorescence on any major class of cells in dissociated cultures of cerebellum or cerebral hemispheres (unpublished observations of K.L.F); thus it is not a general glial marker. It will be of interest to compare the characteristics of this antigen and the recently described surface antigen on human Schwann cells[13].

Since the fibroblasts[3], but not Schwann cells, can be killed by anti-Thy-1 and complement (unpublished observations) it should be possible to remove the fibroblasts from sciatic nerve cultures; alternatively, the Schwann cells could be positively selected by the use of a fluorescence activated cell sorter[14]. In either case, the availability of surface markers for both cell types should prove useful in studies of myelination *in vitro*, and of other interactions with nerve[15] and muscle[16]. Moreover, the availability of one neural cell marker should facilitate the search for others.

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Developmentally regulated lectin in neonatal rat brain

WE report the identification of a developmentally regulated agglutinin of erythrocytes present in neonatal rat brain. Specific inhibition by a number of saccharides with a galactose configuration suggest that multivalent carbohydrate-binding proteins, 'endogenous brain lectins', may be responsible for the observed agglutination activity. We are presently investigating the functional role of this lectin-like activity in brain, since molecules with similar carbohydrate-binding properties seem likely to mediate intercellular recognition and adhesion in certain lower eukaryotic organisms.

The ability of cells to recognise other cells and specifically adhere to them is a central problem in developmental neurobiology and the subject of intense investigation. Moscona[1], more than a decade ago, proposed that specific macromolecules at the cell surface are responsible for specific adhesion. Specific cell recognition and adhesion could then be achieved by a cell-surface macromolecule, a carbohydrate-binding protein[2] or an enzyme[3] with a particular recognition specificity, and a carbohydrate-containing receptor, one or both of which is under a temporal metabolic regulation. The lectin hypothesis of cell recognition and adhesion[2] has gained support in recent studies[4–8] with the cellular slime moulds (for review, see ref. 9). Developmentally regulated carbohydrate-binding proteins, "discoidin"[4] and "pallidin"[6] accumulate on the cell surface of *Dictyostelium discoideum* and *Polysphondylium pallidum* as these species differentiate from a vegetative single cell state to a cohesive aggregation-competent state, and substantial evidence[2,4–8] indicates that these slime mould lectins may mediate intercellular recognition and adhesion by interacting with specific cell-surface receptors[7]. Teichberg *et al.*[10] reported the presence of a β-D-galactoside-binding protein in *Electrophorus electricus* and in a number of mammalian tissues and tissue culture cells, and Nowak *et al.*[11], and Gartner and Podleski[12], have studied a β-D-galactoside binding protein in embryonic chick muscle and myogenic cell lines, a protein, purified and characterised by Den and Malinzak[13].

The functional components of intercellular recognition and adhesion are generally thought to be glycoproteins[14] and in brain tissue in particular, the synaptic specialisation is enriched by carbohydrate-containing macromolecules which may function in synaptogenesis[14,15], in conjunction with carbohydrate-binding molecules of appropriate specificity.

Soluble extracts of developing neonatal rat brain contain a 'lectin-like' agglutination activity which is measured with trypsinised glutaraldehyde-fixed rabbit erythrocytes. In brain extracts prepared from 1–30-d-old rats (Fig. 1) a marked increase in agglutination activity was observed from day 1–10, followed by a decline to undetectable levels between 10 and 21 d. Only trace amounts of activity were found in adult animals. The time course seen in Fig. 1 was reproduced with different sets of brain extracts, and different preparations of rabbit erythrocytes, although the absolute value of the titre varied somewhat with the particular preparation of erythrocytes. Variable agglutinability of erythrocyte preparations could result from individual donor differences or from minor variations in the conditions of trypsinisation or glutaraldehyde fixation. Specific agglutination activity—that is, agglutination activity per mg protein—parallels the curve seen in Fig. 1. The possibility that the observed time course was a function of changes in the extracting reagent (DES) or in the agglutinability of the erythrocyte preparation over the time course of the study was eliminated by preparing extracts at the individual time points, freezing and assaying them together on the same day, or by simultaneously preparing extracts of animals at various ages and assaying on the same day. Mixing experiments in which day 1 and 10 extracts and day 10 and 30 extracts were mixed, incubated and assayed, gave intermediate values suggesting that enzymatic modification in crude soluble extracts was not responsible for the ascending or descending portions of the time course. No agglutination was observed when serum from 10-d-old rats was tested for this activity.

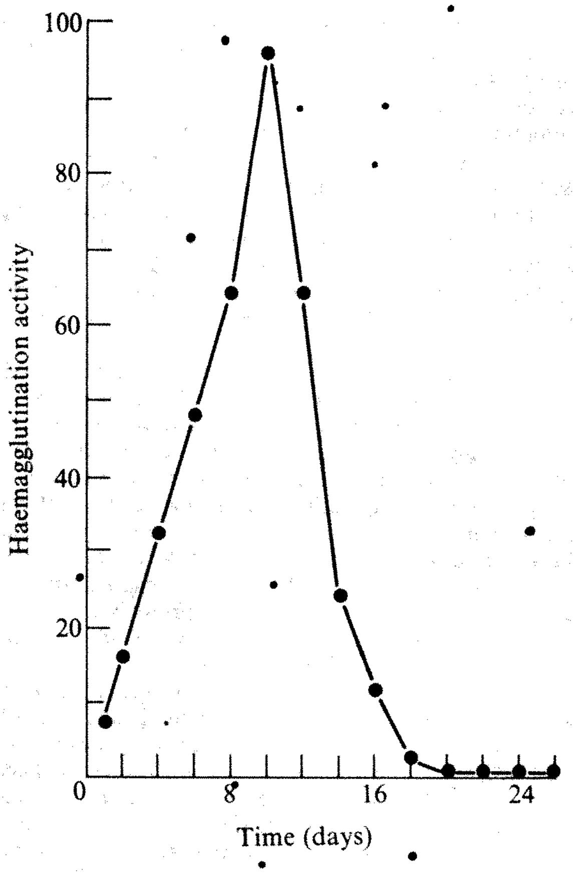

Fig. 1 Sprague–Dawley pups from litters normalised to eight pups per mother at birth, to ensure that all had equal and sufficient nutrition, were killed by decapitation. Brains were homogenised at 4 °C in 4 volumes of freshly prepared 2 mM dithiothreitol, 2 mM EDTA, 0.15 M NaCl, abbreviated DES, in a Potter–Elvejhem tissue homogeniser. Dithiothreitol at 10 mM did not improve the extractability of activity but served to stabilise the activity extracted. The homogenate was centrifuged 60 min at 40,000 r.p.m. and the supernatant assayed for agglutination activity using trypsinised glutaraldehyde-fixed rabbit erythrocytes. Erythrocytes for agglutination assays were prepared from rabbit blood (Colorado Serum Labs) collected in Alsever's solution. Cells were washed 4 times with 5 volumes saline. Erythrocytes were suspended 4% (v/v) in 0.1 M sodium phosphate–0.05 M NaCl, *p*H 7.4, and trypsin from bovine pancreas (Sigma) 0.1 mg ml, was added, and the suspension incubated at 37 °C for 60 min. Cells were then washed 5 times with 10 volumes saline. Washed erythrocytes were suspended in 5 volumes 0.075 M Na, K phosphate–0.075 M NaCl, *p*H 7.2 (PBS 7.2), containing 1% glutaraldehyde (Sigma, grade I), and incubated with shaking for 1 h at room temperature. The cross-linking reaction was terminated by addition of 5 volumes 0.1 M glycine in PBS 7.2 at 4 °C. Erythrocytes were sedimented and washed 3 times with 5 volumes 0.1 M glycine in PBS 7.2. Finally, cells were washed 4 times in 5 volumes cold PBS 7.2. Agglutination activity in brain extracts was measured with trypsinised glutaraldehyde-fixed rabbit erythrocytes as follows. A twofold dilution series of brain extract was made in DES. Either 25 μl PBS 7.2 or 25 μl an appropriate hapten inhibitor in PBS 7.2 was added to wells of a microtitre *V* plate (Cooke Engineering). Aliquots (25 μl), of the serial dilutions of extract were added to wells followed by 25 μl 1% solution bovine serum albumin in 0.15 M NaCl. After gentle mixing, 25 μl of a 2.5% suspension of erythrocytes in PBS 7.2 was added to wells. Plates were shaken vigorously, covered with transparent tape, and left at room temperature for 90 min before agglutination patterns were evaluated. The agglutination endpoint was taken as the first dilution of extract at which erythrocytes settled into a clearly circumscribed dot at the bottom of the well. Agglutination activity is defined as the reciprocal of the endpoint dilution.

A number of other types of erythrocytes were tested for their ability to calibrate agglutination activity. Fresh rabbit erythrocytes were not agglutinable, nor did trypsinisation of fresh erythrocytes render them agglutinable. Fixation with glutaraldehyde or formalin was required to sensitise the rabbit cells to agglutination, whereas trypsinisation before fixation gave 50% higher titres suggesting that such treatment, although insufficient in itself, does increase the agglutinability of fixed cells. Trypsinised formalin-fixed rabbit erythrocytes and trypsinised glutaraldehyde-fixed or formalin-fixed sheep erythrocytes were four times less sensitive to agglutination than the trypsinised glutaraldehyde-fixed rabbit erythrocytes, whereas formalin-treated human type O erythrocytes were eleven times less sensitive.

Agglutination activity of 10-d-old rats was specifically inhibited by a number of monosaccharides and disaccharides with a galactose configuration, in addition to hexosamines, mannose, and a brain glycopeptide fraction[16], as seen in Table 1. This non-dialisable glycopeptide fraction was found to be the most potent inhibitor of agglutination, being nearly 150 times greater on a molar basis than the most effective monosaccharide inhibitor, galactosamine. The glycopeptide fraction, with an average molecular weight of 8,000, was prepared from lipid-extracted whole brain by extensive proteolytic digestion, followed by quantitative precipitation of contaminating glycosaminoglycans with cetylpyridinium chloride[16]. The potent inhibition by hexosamines is interesting in view of the report by Yamada *et al.*[17], that the CSP protein or LETS protein, an agglutinin of formalin fixed erythrocytes, which has been identified as a surface antigen in cultured glial cells[18], was also inhibited by hexosamines. Careful re-examination of the hexosamine inhibition in brain extracts by measuring hexosamine inhibition at various amino sugar concentrations in parallel with equimolar concentrations of NaCl, suggested that the inhibition at high concentrations was predominantly a charge effect, whereas studies at lower hexosamine concentrations revealed a galactosamine-specific inhibition. Agglutination by the brain extract is probably not due to LETS protein since EDTA or EGTA, potent inhibitors of the LETS mediated agglutination[17], were not potent inhibitors of the brain activity. Nevertheless, we cannot absolutely rule out the possibility that the brain activity, in part, is LETS mediated, since a different cell type was used to test agglutination by purified LETS protein. An accurate comparison to the hapten inhibition specificity of the LETS protein, awaits purification of the brain agglutinin.

Although the sugar concentrations required to inhibit agglutination by brain extracts are relatively high, unlike those of many lectins derived from plant sources, a number of lectins do require concentrations in this range. The carbohydrate specificity, although generally galactose specific, is not absolute suggesting that several carbohydrate-binding proteins with distinct specificities may be present in crude extracts from 10-d-old rats.

Table 1 Glycopeptide and sugar effects on agglutination of erythrocytes by brain extracts

Sugar concentrations (mM) that inhibit agglutination by 50%			
Brain glycopeptides	0.1	*N*-acetylglucosamine	>125
Galactosamine	15	*N*-acetylmannosamine	>125
α-lactose	30	Glucose	>125
Thiodigalactoside	30	L-fucose	>125
Mannosamine	30	L-rhamnose	>125
Glucosamine	30	α-methyl-D-galactose	>125
Mannose	60	α-methyl-D-glucose	>125
Galactose	60	α-methyl-D-mannose	>125
N-acetylglactosamine	>125	2-deoxy-D-glucose	>125

Brain extract from 10-d-old rats was assayed for agglutination activity as described in Fig. 1 in the presence of a series of concentrations of various sugars in PBS 7.2 to determine the sugar concentration that would inhibit agglutination activity by 50%. In addition, brain glycopeptides, prepared as described by Simpson *et al.*[16], were tested as inhibitors of agglutination. The molarity of the glycopeptide was based on an average molecular weight of 8,000, determined by gel-filtration on Sephadex G-50.

Extracts containing agglutination activity were subjected to a variety of enzymatic and chemical treatments described in Table 2. The carbohydrate-binding protein was extremely sensitive to trypsin and chymotrypsin and somewhat less sensitive to neuraminidase. No decrease in activity was observed after incubation with a variety of other enzymes. Agglutination activity was quantitatively inactivated by heating at 100° C for 5 min. Mild periodate oxidation in controlled conditions[19] also quantitatively destroyed the agglutination activity. The effects of trypsin, chymotrypsin, neuraminidase and periodate oxidation suggests that both protein and carbohydrate components are in some way required for agglutination.

Table 2 Enzymatic and chemical characterisation of carbohydrate-binding activity in developing rat brain

Enzymatic or chemical treatment	Concentration (μg ml^{-1})	Decrease in activity (%)
Trypsin	0.1	25
	1	100
	10	100
	100	100
Chymotrypsin	0.1	0
	1	100
	10	100
	100	100
Neuraminidase	200	75
	100	50
β-glucosidase	200	0
Collagenase	200	0
Hyaluronidase	200	0
Phospholipase D	200	0
Ribonuclease	200	0
Deoxyribonuclease	200	0
Heat (5 min, 100 °C)	—	100
Sodium periodate (1 h, 4 °C)	0.012 M	100

Rat brain extract from 10-d-old pups was incubated with a series of enzymes at the specified final concentrations for 1 h at 37 °C. Controls without enzyme and without extract were incubated and assayed for agglutination activity in parallel experiments. Trypsin sensitivity was also demonstrated when the action of trypsin was terminated after incubation by addition of excess trypsin soybean inhibitor before assay, or when a Sepharose–trypsin conjugate (Sigma) was used. Also a decrease in agglutination could be demonstrated in experiments using a Sepharose–neuraminidase conjugate (Sigma), although this effect may be due in part to the action of protease contaminates. Mild treatment with sodium periodate in controlled conditions quantitatively inhibited agglutination activity. Incubation conditions used were designed for the selective oxidation of terminal sialic acid residues[19].

The heat lability, enzyme studies, and the hapten inhibition specificity suggest that the observed agglutination is not a result of the adventitious association of glycosaminoglycans or nucleic acids with erythrocytes. The activity we have identified in rat brain does not seem to be related to an agglutinin of formalin treated chick erythrocytes which accumulates in chick brain during embryonic development[20]. Agglutination produced by this extremely large proteoglycan-like complex cannot be inhibited by specific saccharides and its activity is insensitive to boiling.

The peak of agglutination activity (Fig. 1) is coincident with the time course of maximum synapse formation in developing rat brain cortex[21]. It is also interesting that the agglutination activity seems to parallel the temporal course of maximum *in vitro* cohesiveness of homotypic interactions of disassociated neuronal cells[22,23]. These parallels are at best suggestive, and further experiments are required to implicate functionally this agglutination activity in the specific cellular interactions required for synapotogenesis and neuronal development.

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Soluble extracts of developing neonatal rat brain contain a 'lectin-like' agglutination activity which is measured with trypsinised glutaraldehyde-fixed rabbit erythrocytes. In brain extracts prepared from 1–30-d-old rats (Fig. 1) a marked increase in agglutination activity was observed from day 1–10, followed by a decline to undetectable levels between 10 and 21 d. Only trace amounts of activity were found in adult animals. The time course seen in Fig. 1 was reproduced with different sets of brain extracts, and different preparations of rabbit erythrocytes, although the absolute value of the titre varied somewhat with the particular preparation of erythrocytes. Variable agglutinability of erythrocyte preparations could result from individual donor differences or from minor variations in the conditions of trypsinisation or glutaraldehyde fixation. Specific agglutination activity—that is, agglutination activity per mg protein—parallels the curve seen in Fig. 1. The possibility that the observed time course was a function of changes in the extracting reagent (DES) or in the agglutinability of the erythrocyte preparation over the time course of the study was eliminated by preparing extracts at the individual time points, freezing and assaying them together on the same day, or by simultaneously preparing extracts of animals at various ages and assaying on the same day. Mixing experiments in which day 1 and 10 extracts and day 10 and 30 extracts were mixed, incubated and assayed, gave intermediate values suggesting that enzymatic modification in crude soluble extracts was not responsible for the ascending or descending portions of the time course. No agglutination was observed when serum from 10-d-old rats was tested for this activity.

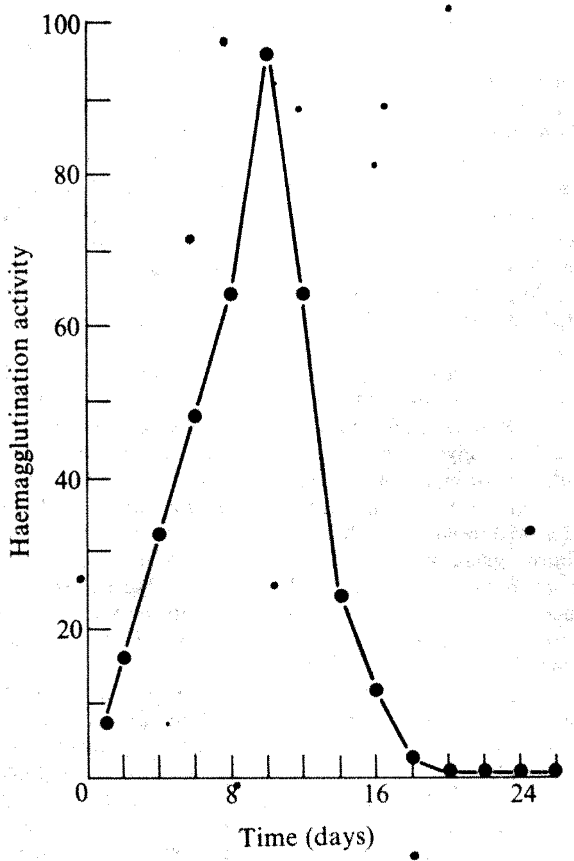

Fig. 1 Sprague–Dawley pups from litters normalised to eight pups per mother at birth, to ensure that all had equal and sufficient nutrition, were killed by decapitation. Brains were homogenised at 4 °C in 4 volumes of freshly prepared 2 mM dithiothreitol, 2 mM EDTA, 0.15 M NaCl, abbreviated DES, in a Potter–Elvejhem tissue homogeniser. Dithiothreitol at 10 mM did not improve the extractability of activity but served to stabilise the activity extracted. The homogenate was centrifuged 60 min at 40,000 r.p.m. and the supernatant assayed for agglutination activity using trypsinised glutaraldehyde-fixed rabbit erythrocytes. Erythrocytes for agglutination assays were prepared from rabbit blood (Colorado Serum Labs) collected in Alsever's solution. Cells were washed 4 times with 5 volumes saline. Erythrocytes were suspended 4% (v/v) in 0.1 M sodium phosphate–0.05 M NaCl, *p*H 7.4, and trypsin from bovine pancreas (Sigma) 0.1 mg ml, was added, and the suspension incubated at 37 °C for 60 min. Cells were then washed 5 times with 10 volumes saline. Washed erythrocytes were suspended in 5 volumes 0.075 M Na, K phosphate–0.075 M NaCl, *p*H 7.2 (PBS 7.2), containing 1% glutaraldehyde (Sigma, grade I), and incubated with shaking for 1 h at room temperature. The cross-linking reaction was terminated by addition of 5 volumes 0.1 M glycine in PBS 7.2 at 4 °C. Erythrocytes were sedimented and washed 3 times with 5 volumes 0.1 M glycine in PBS 7.2. Finally, cells were washed 4 times in 5 volumes cold PBS 7.2. Agglutination activity in brain extracts was measured with trypsinised glutaraldehyde-fixed rabbit erythrocytes as follows. A twofold dilution series of brain extract was made in DES. Either 25 µl PBS 7.2 or 25 µl an appropriate hapten inhibitor in PBS 7.2 was added to wells of a microtitre *V* plate (Cooke Engineering). Aliquots (25 µl), of the serial dilutions of extract were added to wells followed by 25 µl 1% solution bovine serum albumin in 0.15 M NaCl. After gentle mixing, 25 µl of a 2.5% suspension of erythrocytes in PBS 7.2 was added to wells. Plates were shaken vigorously, covered with transparent tape, and left at room temperature for 90 min before agglutination patterns were evaluated. The agglutination endpoint was taken as the first dilution of extract at which erythrocytes settled into a clearly circumscribed dot at the bottom of the well. Agglutination activity is defined as the reciprocal of the endpoint dilution.

A number of other types of erythrocytes were tested for their ability to calibrate agglutination activity. Fresh rabbit erythrocytes were not agglutinable, nor did trypsinisation of fresh erythrocytes render them agglutinable. Fixation with glutaraldehyde or formalin was required to sensitise the rabbit cells to agglutination, whereas trypsinisation before fixation gave 50% higher titres suggesting that such treatment, although insufficient in itself, does increase the agglutinability of fixed cells. Trypsinised formalin-fixed rabbit erythrocytes and trypsinised glutaraldehyde-fixed or formalin-fixed sheep erythrocytes were four times less sensitive to agglutination than the trypsinised glutaraldehyde-fixed rabbit erythrocytes, whereas formalin-treated human type O erythrocytes were eleven times less sensitive.

Agglutination activity of 10-d-old rats was specifically inhibited by a number of monosaccharides and disaccharides with a galactose configuration, in addition to hexosamines, mannose, and a brain glycopeptide fraction[16], as seen in Table 1. This non-dialisable glycopeptide fraction was found to be the most potent inhibitor of agglutination, being nearly 150 times greater on a molar basis than the most effective monosaccharide inhibitor, galactosamine. The glycopeptide fraction, with an average molecular weight of 8,000, was prepared from lipid-extracted whole brain by extensive proteolytic digestion, followed by quantitative precipitation of contaminating glycosaminoglycans with cetylpyridinium chloride[16]. The potent inhibition by hexosamines is interesting in view of the report by Yamada *et al.*[17], that the CSP protein or LETS protein, an agglutinin of formalin fixed erythrocytes, which has been identified as a surface antigen in cultured glial cells[18], was also inhibited by hexosamines. Careful re-examination of the hexosamine inhibition in brain extracts by measuring hexosamine inhibition at various amino sugar concentrations in parallel with equimolar concentrations of NaCl, suggested that the inhibition at high concentrations was predominantly a charge effect, whereas studies at lower hexosamine concentrations revealed a galactosamine-specific inhibition. Agglutination by the brain extract is probably not due to LETS protein since EDTA or EGTA, potent inhibitors of the LETS mediated agglutination[17], were not potent inhibitors of the brain activity. Nevertheless, we cannot absolutely rule out the possibility that the brain activity, in part, is LETS mediated, since a different cell type was used to test agglutination by purified LETS protein. An accurate comparison to the hapten inhibition specificity of the LETS protein, awaits purification of the brain agglutinin.

Although the sugar concentrations required to inhibit agglutination by brain extracts are relatively high, unlike those of many lectins derived from plant sources, a number of lectins do require concentrations in this range. The carbohydrate specificity, although generally galactose specific, is not absolute suggesting that several carbohydrate-binding proteins with distinct specificities may be present in crude extracts from 10-d-old rats.

Table 1 Glycopeptide and sugar effects on agglutination of erythrocytes by brain extracts

Sugar concentrations (mM) that inhibit agglutination by 50%			
Brain glycopeptides	0.1	*N*-acetylglucosamine	> 125
Galactosamine	15	*N*-acetylmannosamine	> 125
α-lactose	30	Glucose	> 125
Thiodigalactoside	30	L-fucose	> 125
Mannosamine	30	L-rhamnose	> 125
Glucosamine	30	α-methyl-D-galactose	> 125
Mannose	60	α-methyl-D-glucose	> 125
Galactose	60	α-methyl-D-mannose	> 125
N-acetylglactosamine	> 125	2-deoxy-D-glucose	> 125

Brain extract from 10-d-old rats was assayed for agglutination activity as described in Fig. 1 in the presence of a series of concentrations of various sugars in PBS 7.2 to determine the sugar concentration that would inhibit agglutination activity by 50%. In addition, brain glycopeptides, prepared as described by Simpson *et al.*[16], were tested as inhibitors of agglutination. The molarity of the glycopeptide was based on an average molecular weight of 8,000, determined by gel-filtration on Sephadex G-50.

Extracts containing agglutination activity were subjected to a variety of enzymatic and chemical treatments described in Table 2. The carbohydrate-binding protein was extremely sensitive to trypsin and chymotrypsin and somewhat less sensitive to neuraminidase. No decrease in activity was observed after incubation with a variety of other enzymes. Agglutination activity was quantitatively inactivated by heating at 100° C for 5 min. Mild periodate oxidation in controlled conditions[19] also quantitatively destroyed the agglutination activity. The effects of trypsin, chymotrypsin, neuraminidase and periodate oxidation suggests that both protein and carbohydrate components are in some way required for agglutination.

Table 2 Enzymatic and chemical characterisation of carbohydrate-binding activity in developing rat brain

Enzymatic or chemical treatment	Concentration (μg ml^{-1})	Decrease in activity (%)
Trypsin	0.1	25
	1	100
	10	100
	100	100
Chymotrypsin	0.1	0
	1	100
	10	100
	100	100
Neuraminidase	200	75
	100	50
β-glucosidase	200	0
Collagenase	200	0
Hyaluronidase	200	0
Phospholipase D	200	0
Ribonuclease	200	0
Deoxyribonuclease	200	0
Heat (5 min, 100 °C)	—	100
Sodium periodate (1 h, 4 °C)	0.012 M	100

Rat brain extract from 10-d-old pups was incubated with a series of enzymes at the specified final concentrations for 1 h at 37 °C. Controls without enzyme and without extract were incubated and assayed for agglutination activity in parallel experiments. Trypsin sensitivity was also demonstrated when the action of trypsin was terminated after incubation by addition of excess trypsin soybean inhibitor before assay, or when a Sepharose–trypsin conjugate (Sigma) was used. Also a decrease in agglutination could be demonstrated in experiments using a Sepharose–neuraminidase conjugate (Sigma), although this effect may be due in part to the action of protease contaminates. Mild treatment with sodium periodate in controlled conditions quantitatively inhibited agglutination activity. Incubation conditions used were designed for the selective oxidation of terminal sialic acid residues[19].

The heat lability, enzyme studies, and the hapten inhibition specificity suggest that the observed agglutination is not a result of the adventitious association of glycosaminoglycans or nucleic acids with erythrocytes. The activity we have identified in rat brain does not seem to be related to an agglutinin of formalin treated chick erythrocytes which accumulates in chick brain during embryonic development[20]. Agglutination produced by this extremely large proteoglycan-like complex cannot be inhibited by specific saccharides and its activity is insensitive to boiling.

The peak of agglutination activity (Fig. 1) is coincident with the time course of maximum synapse formation in developing rat brain cortex[21]. It is also interesting that the agglutination activity seems to parallel the temporal course of maximum *in vitro* cohesiveness of homotypic interactions of disassociated neuronal cells[22,23]. These parallels are at best suggestive, and further experiments are required to implicate functionally this agglutination activity in the specific cellular interactions required for synapotogenesis and neuronal development.

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Effects of glycolipids on *in vitro* development of neuromuscular junction

THE nervous system has a unique distribution of glycolipids[1], which changes markedly during growth of the animals[2] and in various inherited metabolic disorders[3]. No physiological roles for glycolipids in neural function are known, however. But, in nonneural cells glycolipids of the cell membrane are essential for cell recognition and interaction[4]. For example, some gangliosides act as receptors for various bacterial toxins[5] and globoside has been identified as the human P blood type antigen[6]. When administered in the medium, ganglioside can be incorporated into cell membranes and function as toxin receptors[5,7]. Ganglioside added to the medium competes with membrane receptors by binding to the toxins[5]. We reported previously[8,9] that the effects of some drugs and sera on the formation of neuromuscular junction in culture could be evaluated by recording the endplate potential (e.p.p.) from muscle cells combined with the spinal cord. We describe here the effect of glycolipids on the development of neuromuscular junction in culture of skeletal muscle and the spinal cord. Globoside had biphasic effects, an inhibitory effect at the higher concentration and a facilitatory one at the lower concentration or on brief exposure at the beginning of the cultivation. Our evidence suggests that glycolipids are involved in formation of the neuromuscular junction.

Pectoral muscles and spinal cords were obtained from 10–11-d and 5–7-d chick embryos (White Leghorn) respectively. The muscles were dissociated to single cells by incubation in 0.25% trypsin and $2.5–5 \times 10^5$ cells were plated into a plastic culture dish (35 mm diameter, Falcon). After 2 or 3 d of culturing muscle cells alone, fragments (about 15 pieces) of the spinal cord were added to each dish. The cord fragments were obtained by slicing the whole length of the hemisected spinal cord. The culture medium was Eagle's minimum essential medium (MEM, Nissui) supplemented with 10% foetal calf serum, 5% chick serum (both Flow Labs.), 6 mg ml^{-1} glucose and 0.06 mg ml^{-1} kanamycin. The cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. At the time of plating the cord the medium was renewed and glycolipid (Table 1) was added. In some experiments globoside was added according to the schedule described in Table 3. Glycolipids were prepared as before[10–13]. Galactosylceramide, sulphatide and gangliosides (a mixture of G_{M1}–G_{T1} and G_{M1}) were from bovine brain[10,11]. Glucosylceramide was isolated from the spleen of a patient with Gaucher's disease, and purified by Florisil and silicic acid column chromatography. Haematoside (*N*-glycolylneuraminyl-galactosyl-glucosyl-ceramide) and globoside (*N*-acetylgalactosaminyl-galactosyl-galactosyl-glucosyl-ceramide) were isolated from equine and human erythrocytes respectively[12,13]. The sugar chain of globoside (*N*-acetylgalactosaminyl-galactosyl-galactosyl-glucose) was obtained by ozonolysis and mild alkali treatment of globoside[14,15], and further purified by charcoal column chromatography. The purity of the oligosaccharide was checked by gas–liquid chromatography[15].

After further 2 d of coculture, the culture dish was mounted on the stage of an inverted phase-contrast microscope and membrane potentials of myotubes (multinucleated muscle cells) were recorded intracellularly with a KCl-filled electrode (resistance 15–20 MΩ). The experiments were usually performed in the fresh MEM without serum and glycolipid at 35–38 °C. The presence of glycolipid in the medium, however, did not interfere with measurement of electrophysiological parameters, and did not alter the results.

A proportion of myotubes, cultured for 2 d in combination with the spinal cord explant, spontaneously generated e.p.ps (ref. 8) (Fig. 1). The e.p.ps varied in amplitude from myotube to myotube (range 1–10 mV) and in frequency (0.5–30 per s). As in the previous studies[8,9], the presence of spontaneous e.p.ps was regarded as indicating the formation of a neuro-

Table 1 Spontaneous e.p.p.s in myotubes cultured for 2 d with the spinal cord in the presence of glycolipids

Glycolipid	Concentration (mM)	No. of myotubes tested	Myotubes with e.p.ps (%)
None (control)		435	55
Galactosylceramide	0.5	60	63*
Glucosylceramide	0.5	57	56*
Haematoside	0.5	56	41*
Sulphatide	0.05	62	63*
Gangliosides (G_{M1}–G_{T1})	0.5	88	45*
G_{M1}	0.5	57	21†
Globoside	0.5	52	23†

* $P > 0.05$, compared with control by χ^2 test on 2×2 contingency tables.
† $P < 0.01$.

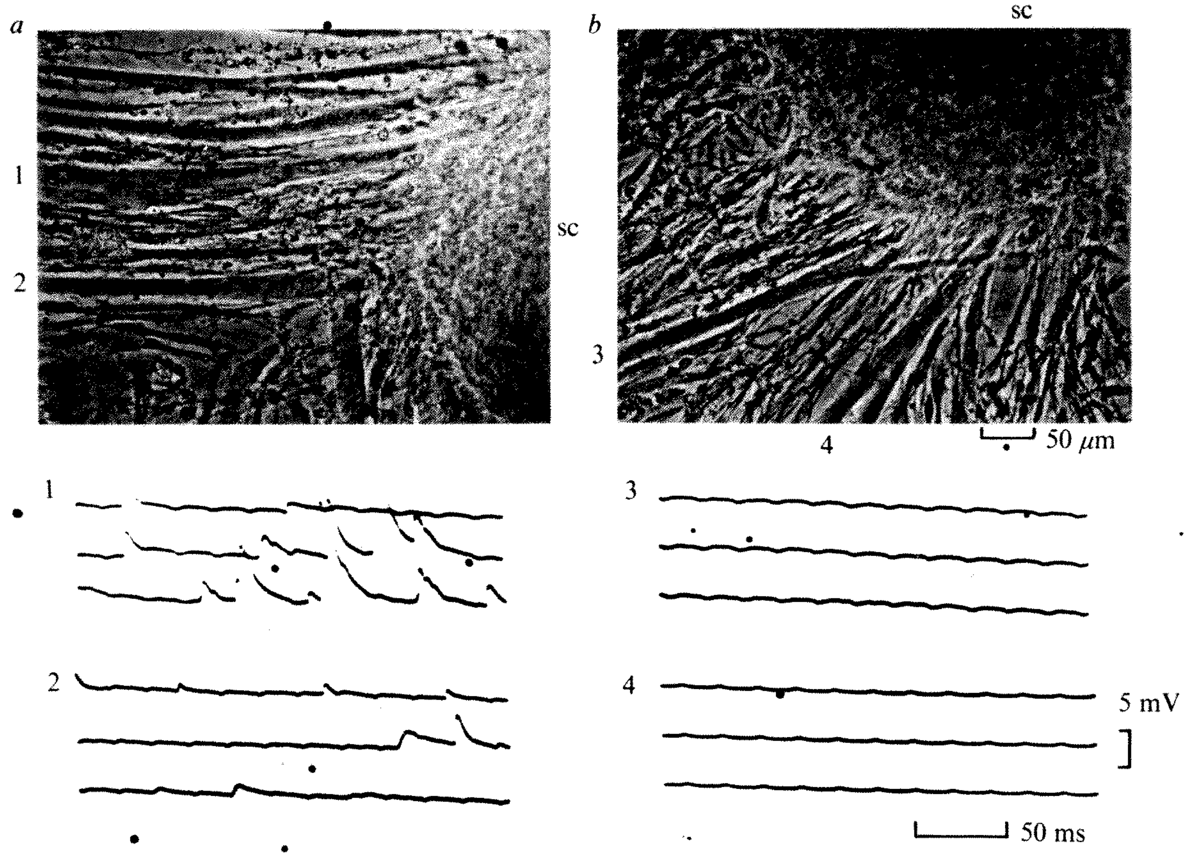

Fig. 1 Photomicrographs of cultures of skeletal muscle and the spinal cord (sc) from the chick embryo, incubated for 2 d. *a*, Without addition of glycolipid, and *b*, in the presence of 0.25 mM globoside. Potential records 1–4 were obtained from myotubes 1–4 respectively, as indicated in the photomicrographs. Spontaneous e.p.ps were obtained from myotubes 1 and 2 but not from 3 and 4.

muscular junction. The myotubes which were partially covered with the spinal cord explants or with numerous nerve processes were chosen for electrode penetration. Myotubes with a recorded resting potential lower than –40 mV were not included in the study. Four independent myotubes were sampled for each explant and 10–12 myotubes were studied in each culture dish. Percentages of myotubes generating spontaneous e.p.ps were compared for seven glycolipid samples (Table 1) at a concentration of 0.5 mM, except for sulphatide. Sulphatide at concentrations over 0.1 mM was toxic to growth of muscle cells and nerve processes, so the effect on junction formation was studied at 0.05 mM. The results are summarised in Table 1. Compared with the control cultures grown without glycolipids, galactosylceramide and sulphatide showed high values and glucosylceramide was without change. Cultures grown with haematoside, the mixture of gangliosides, G_{M1} and globoside were lower in percentages of myotubes with e.p.ps than controls. This difference was statistically significant only for globoside and G_{M1}, although growth of nerve and muscle did not seem to be impaired (Fig. 1 and see later). Further analyses were mainly made using globoside.

Effects of globoside varied with concentration (Fig. 2); an inhibitory effect was observed at higher concentrations (0.25–0.5 mM), but junction formation was augmented at lower concentrations (8–63 μM). Biphasic effects were also observed with the sugar chain of globoside, although the transition from facilitation to depression occurred at a higher concentration than with globoside (Fig. 2). The experiments to determine whether there was a direct effect of globoside on the growth of nerve and muscle are summarised in Table 2. Outgrowth of nerve processes was not affected. High concentrations (0.25–0.5 mM) of globoside sometimes impaired the growth of myotubes but resting potential, input resistance and acetylcholine sensitivity were the same in cells cultured with and without globoside. Globoside thus has a rather selective effect on the junction formation. The incidence of junction formation was low in the presence of 0.5 mM G_{M1}, but high at 0.25 mM (myotubes with e.p.ps constituting 72% of 40 cells tested).

Globoside was also added to cultures for various times as shown in Table 3, at a constant concentration of 0.25 mM. In contrast with depression induced by exposure to globoside throughout the culture period (48 h), exposure to globoside

Fig. 2 Effects of globoside (○) and its sugar chain (●) on the development of neuromuscular junction in culture. Myotubes were cultured with the spinal cord for 2 d in the presence of various concentrations of test substances. Ordinate shows percentage of myotubes which were spontaneously generating e.p.ps. A dotted line indicates level of the control (55%, Table 1). The numbers of myotubes tested are shown in parentheses.

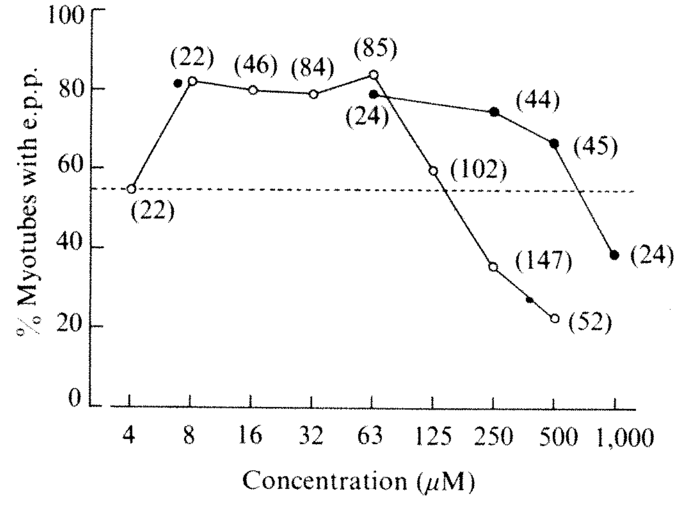

Table 2 Globoside effect on nerve and muscle cell culture growth

Globoside concentration (mM)	0	0.05	0.25
Process outgrowth from the spinal cord explants			
Density	1.7±0.11	1.4±0.17	1.6±0.13
Length	2.1±0.12 (46)	2.0±0.23 (24)	1.9±0.17 (28)
Electrical properties of myotubes			
Resting potential (mV)	−56.5±18.1 (184)	−57.9±19.6 (71)	−54.7±20.9 (155)
Input resistance (mΩ)	2.5±1.0 (3)		2.6±0.7 (3)
Acetylcholine sensitivity (mV nC^{-1})	425±436 (6)		483±287 (13)

Explants of the spinal cord from 6 d chick embryo were cultured for 2 d without muscle cells, with or without globoside. The extent of process outgrowth in fibre density and length was rated on an arbitrary scale of 0–3, a score of 3 indicating the maximum (modified from Mizel and Bamburg[17]). Electrical properties of myotubes were obtained from the same cultures as for the junction formation. Input resistance was measured by two intracellular electrode method. Acetylcholine was applied electrophoretically through micropipettes. In parentheses are numbers of the spinal cord explants or myotubes tested. No statistical significance was found when compared with the control (globoside, 0 mM).

during the first 12–24 h markedly potentiated junction formation. In these experiments the culture was repeatedly washed after the exposure, because even at a very low concentration globoside would affect the succeeding culture (see Fig. 2). Exposure for the first 6 h in a small number of experiments also showed mild augmentation of junction formation (not shown). Table 3 also shows that junction formation was low when globoside was applied later (12–24 h after plating the spinal cord). In a further experiment (not shown) globoside was applied to muscle culture for 24 h before the spinal cord was added. When studied after 2 d of coculture with the cord, e.p.ps were observed in 40% of 50 myotubes. Growth of myotubes was slightly impaired in these cultures. This suggests that the facilitatory effect was exerted on the nerve rather than on the muscle cells.

Table 3 Effect of time of globoside application (0.25 mM) on development of the e.p.ps in 48-h culture of muscle and the spinal cord

Exposure time (h)	No. of myotubes tested	Myotubes with e.p ps (%)
0–48	147	36
0–12	86	85*
0–24	95	78*
12–48	72	40†
24–48	89	44†

*$P < 0.01$ when compared with 0–48-h value by χ^2 test on 2×2 contingency tables.
†$P > 0.05$.

Judging from the onset of appearance of the spontaneous e p.p.s, neuromuscular junctions in these cultures were formed 12–48 h after plating the spinal cord (most being formed at 20–30 h). The fact that junction formation was stimulated by globoside during first 12 h of exposure suggests that, before junction formation, globoside or its sugar chain induces changes in nerve and muscle which resulted in subsequent facilitation of junction formation. Globoside is probably incorporated into cell membrane in a way analogous to that of ganglioside[5,7], although incorporation of globoside has not been studied. The globoside sugar chain, however, is probably not incorporated as a constituent of membrane globoside (as it is a saccharide, and not a glycolipid). Hence the facilitatory effect of globoside and its sugar chain may not necessarily be accounted for by an increased level of membrane globoside.

High concentrations of globoside were inhibitory when added at the time of the junction formation (12–48 h after plating of the spinal cord). It is possible that the inhibitory effect may overcome the facilitatory one which has been exerted in advance.

Development of the chick embryo neuromuscular junction *in vitro* was thus markedly affected by globoside and G_{M1}. Further investigation is required to elucidate the mechanism of their action and also to find their role in the development of the neuromuscular system *in vivo*. The glycolipid composition of skeletal muscle is quite different from that of the nervous tissue[16]. The effects of other glycolipids on junction formation will be studied later.

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Antipsychotics block muscarinic acetylcholine receptor-mediated cyclic GMP formation in cultured mouse neuroblastoma cells

ANTIPSYCHOTIC drugs (for example, phenothiazines, butyrophenones) have side effects similar to those of atropine[1], suggesting that they block the muscarinic acetylcholine receptor. Receptor-binding studies with radioactively-labelled ligands have been used to study the muscarinic acetylcholine receptor and to show that antipsychotic drugs vary in their potency at displacing the ligand from binding sites in rat brain homogenates[2,3] In all receptor binding studies it is essential to demonstrate that binding data in fact reflect a drug-receptor interaction in a pharmacological sense[4] These correlations are ideally determined using the same tissue for binding and for biological studies such as was done for certain polypeptide hormone receptors[4] and for the β-

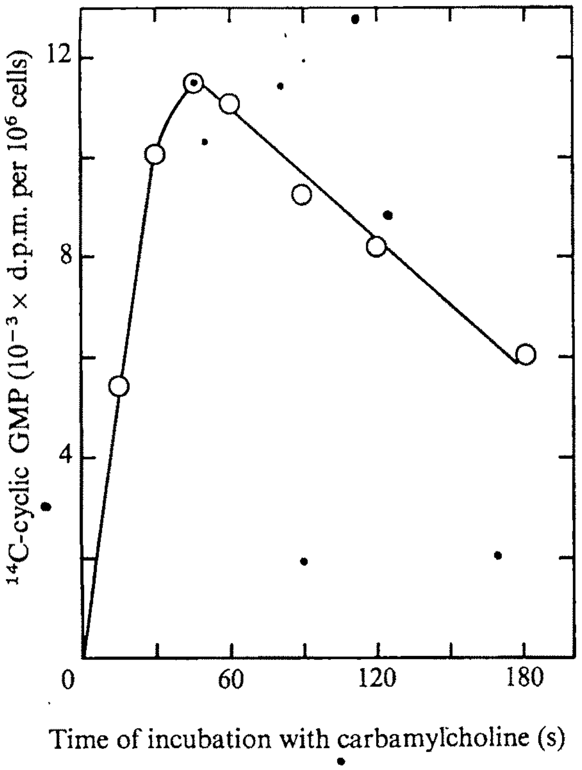


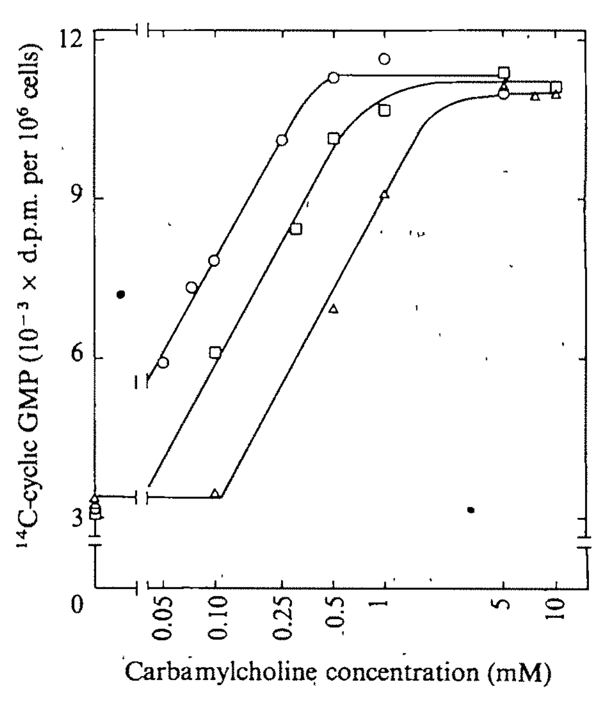
Fig. 1 The effect of carbamylcholine on cyclic GMP formation in cultured mouse neuroblastoma cells. Clone N1E-115 cells were cultured in Dulbecco–Vogt modification of Eagle's medium (high glucose, no pyruvate, Grand Island Biological) supplemented with 10% (v/v) foetal calf serum without antibiotics at 37 °C in an atmosphere of 10% CO_2 and 90% humidified air Cells were grown to confluency in flasks (75 cm² per 250 ml) with 20 ml of medium and maintained in stationary phase (requiring daily medium change) for 6 d before assay For the assay cells were dissociated from the surface of flasks by incubation in a modified Puck's D_1 solution[8], collected by low-speed centrifugation (500 r p m in Damon/IEC CRU-5000 centrifuge) and resuspended at a density of 8 × 10^6 cells per ml in 2.0 ml of phosphate buffered saline (PBS)-HEPES solution containing 110 mM NaCl, 5 3 mM KCl, 1 8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2 0 mM Na_2HPO_4, 25 mM glucose, 50 mM sucrose and 20 mM HEPES *N*-2-hydroxyethyl-piperazine-N_2-ethane titrated to *p*H 7 4 with 1 N NaOH The osmolality of this solution was 335–340 mOsm The cell suspension was then placed in a 25-ml Erlenmeyer flask to which was added U ^{14}C-GTP (specific activity 523 mCi mmol^{-1}) to give a final concentration of 5 μM (1 25 μCi ml^{-1}), and rotated at 37 °C for 45 min at 80 r p m. Cells were then collected by low-speed centrifugation, washed three times with 10 ml of PBS-HEPES to remove extracellular radioactivity, and resuspended in 5 ml of PBS-HEPES containing 0 5 mM IBMX (Aldrich) The radioactive-cell suspension was then distributed in 250-μl aliquots into wells of a multi-well tray and incubated at 37 °C for 30 min at 80 oscillations per min in a shaker bath. A solution of carbamylcholine (Sigma) was then added in 50 μl to give a final concentration of 1 mM. At the indicated times the reaction was stopped by the addition of 100 μl of ice-cold 50% (w/v) trichloroacetic acid (TCA) The TCA solution in each well was then sonicated briefly (3 s at setting no 3, Ultrasonic Cell Disrupter, Kontes Glass) before the addition 0 02 μCi of 8-^{3}H-cyclic GMP as internal standard Each sample was then placed over an 0 8 × 8 cm column of Dowex 50 (AG 50 W-X2, 200–400 mesh, Bio-Rad) which had been washed with water Each well was rinsed once with 500 μl of 5% TCA and this wash was placed over the column. Radioactive cyclic GMP was isolated from each column as described by Fujimoto *et al*[9] Recovery of ^{3}H-cyclic GMP internal standard averaged 86% The ^{14}C-radioactivity in the fraction of the column containing ^{3}H-cyclic GMP was identified as ^{14}C-cyclic GMP by high voltage electrophoresis on Whatman No 1 paper (25 mM NH_4HCO_3 buffer, 1 kV, 5 mA for 60 min) and thin-layer chromatography (PEI-cellulose in two solvent systems *n*-propanol 1 N LiCl (1/1) and 2 N formic acid/2 M LiCl (1/1) Of the recovered ^{14}C-radioactivity, 86–94% electrophoresed or chromatographed with authentic cyclic GMP (Sigma) All time points were determined in duplicate. Corrections were made for recovery of the internal standard and the zero-time point value (5,800 d p m per 10^6 cells) was subtracted from each time point

adrenergic receptor[5]. For the muscarinic acetylcholine receptor of nervous tissue, however, a direct correlation of binding data with pharmacological data has not been shown[6]. Thus, there remains a question whether the displacement by antipsychotic drugs of a radioactively-labelled muscarinic receptor antagonist from brain homogenates reflects blockade by these drugs of this receptor. We report here that antipsychotic drugs block the muscarinic acetylcholine receptor in cultured nerve cells.

For these studies we developed a technique to assay rapidly muscarinic acetylcholine receptor-mediated formation of cyclic GMP in intact cells of adrenergic mouse neuroblastoma clone N1E-115. We have studied this clone in cell culture as a model for the adrenergic neurone using biochemical, pharmacological and electrophysiological techniques[7]. The assay for cyclic GMP (to be described in detail elsewhere) involves radioactively-labelling intracellular pools of GTP and isolation of radioactively-labelled cyclic GMP over a cation exchange resin column

The formation of ^{14}C-cyclic GMP from ^{14}C-GTP in living mouse neuroblastoma cells occurred rapidly after addition of carbamylcholine to the cells (Fig. 1). The peak of ^{14}C-cyclic GMP was at about 45 s in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (Fig. 1) and at about 30 s in the absence of IBMX (data not shown). Since stimulation of the formation of radioactively-labelled cyclic GMP in intact clone N1E-115 cells was inhibited by atropine ($K_B = 2 \times 10^{-9}$ M, E.R , unpublished data) and unaffected by hexamethonium (0.1 mM, E.R., unpublished data) we concluded that this effect was mediated through the muscarinic acetylcholine receptor. Others have found similar results with this clone[10], autonomic ganglia and brain tissue[11] A possible role of muscarinic cholinergic regulation of cyclic GMP in synaptic transmission has been proposed[11].

Fig. 2 Effect of an antipsychotic drug on the carbamylcholine dose–response curve. Mouse neuroblastoma clone N1E-115 (subculture 14) was assayed for carbamylcholine-stimulated cyclic GMP formation as described in the legend to Fig 1 except for the following modifications. Cells in PBS-HEPES with 0 5 mM IBMX were distributed in 250 μl aliquots to each well, 30 μl of PBS-HEPES was added with or without thioridazine (Sandoz) and the cells were incubated for 30 min before addition of carbamylcholine in 20 μl There were approximately 3 1 × 10^5 cells and 224 μg of protein per well. Cell counts were determined with Coulter counter; protein was determined by the modification of the method of Lowrey *et al*[13], using bovine serum albumin as standard ○, No antagonist, □, thioridazine 1 × 10^{-7} M; △, 5 × 10^{-7} M thioridazine



Dose–response curves for carbamylcholine (Fig 2) were shifted to the right in the presence of antipsychotic drugs (as for example, by the piperidine phenothiazine, thioridazine, Fig. 2). The parallel shift of the dose–response curve indicates competitive inhibition and allows the determination of the equilibrium dissociation constant for the antagonist–receptor complex from the equation $K_B = (DR - 1)/[B]$ where DR = dose ratio and $[B]$ = concentration of antagonist[13,14]. In this manner, K_B values for a number of antipsychotic drugs were determined (Table 1). The potency of these antipsychotic drugs varied over a broad range Thus, the most potent drug, clozapine (a dibenzodiazepine) was about 10^4 times more active than the least potent drug prochlorperazine (a phenothiazine) The rank order of potency of these drugs in our system was much the same as that obtained using receptor-binding studies with rat brain homogenates[2,3]. Thus, using a biological assay for the muscarinic acetylcholine receptor, we have demonstrated that antipsychotic drugs competitively block this receptor, thereby blocking muscarinic receptor-mediated formation of cyclic GMP.

Table 1 Equilibrium dissociation constants (K_B) for antipsychotic drugs and the muscarinic acetylcholine receptor

Compound	(K_B) (M)
Clozapine	3×10^{-9}
Thioridazine	6×10^{-8}
Mesoridazine	6×10^{-8}
Promazine	2×10^{-6}
Chlorpromazine	2×10^{-6}
Fluphenazine	2×10^{-6}
Perphenazine	4×10^{-6}
Acetophenazine	4×10^{-6}
Haloperidol	7×10^{-6}
Trifluoperazine	2×10^{-5}
Prochlorperazine	4×10^{-5}

Each compound was tested at 2 different concentrations in a manner similar to that for Fig 2 K_B values were calculated from the shift in the dose–response curves as described in the text Average values are reported

It is likely that *in vivo* antipsychotic drugs block both acetylcholine-stimulated guanylate cyclase activity and dopamine-stimulated adenylate cyclase activity. Effects of antipsychotic drugs on receptor-mediated formation of both cyclic GMP and cyclic AMP may relate to the therapeutic effects as well as the side effects (such as drug-induced Parkinsonism and tardive dyskinesia) of these compounds

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Response of acetylcholine receptors to rapid photochemically produced increases in agonist concentration

SYNAPTIC transmission to muscle fibres and electroplaques takes place on a millisecond timescale To study the molecular events leading to activation of the cholinergic receptor channel, we have developed a method to produce similarly rapid, spatially uniform steps of agonist concentration near intact postsynaptic membranes. *Electrophorus* electroplaques in a voltage-clamp apparatus were exposed to a solution containing a photochromic compound Initially the compound was in a predominantly *cis* form, which had little effect on the membrane. During a brief light flash, some of the compound was isomerised to the *trans* isomer, which is a cholinergic agonist. As a result the membrane conductance increased along a timecourse which reflects the rate processes underlying activation of the cholinergic receptor channel.

Bartels *et al.*[1] first described the photochemistry and pharmacology of 3,3'-*bis*-[α-(trimethylammonium)methyl]azobenzene (*bis*-Q, see Fig. 1) In confirmation of their results, we have found that a curare-sensitive conductance is induced in the innervated membrane by bath application of solutions containing *trans-bis*-Q (ref 2) We have also exploited the fact that for a given concentration of any agonist the steady-state number of open receptor channels increases as the voltage is made more negative After a voltage jump the agonist-induced current relaxes exponentially to a new equilibrium value, at low agonist concentrations the relaxation time constant equals the lifetime of the elementary change ('channel') caused by agonist[3–6] With *trans-bis*-Q, this time constant was about 11 ms at 10 °C and − 150 mV, and about 2.5 ms at 24 °C and the same voltage. Roughly equal values are obtained with acetylcholine (ACh) itself in similar conditions

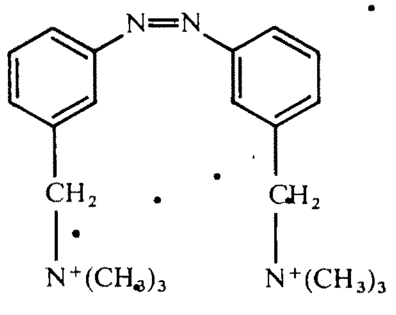
Many quaternary ammonium compounds possessing aromatic groups produce an apparent non-competitive, voltage-dependent blockage of ACh receptors[2,7], but such effects are small or absent with *bis*-Q at the concentrations used here

We have also confirmed the observation[1] that the *cis* isomer has much less effect on ACh receptors, although its precise pharmacology is uncertain because it has not yet been isolated in pure form The *cis* and *trans* isomers are stable in the dark but readily interconvert when exposed to light of appropriate wavelengths (Fig 1). This property affords the opportunity to observe the response to rapid 'agonist concentration jumps'.

Before such experiments the *bis*-Q solutions were converted to a predominantly *cis* form by exposure to 360-nm light until no further changes in optical density were noted at 318 nm. Bartels *et al*[1] estimated that the resulting 'photostationary' state consists of 85% *cis* and 15% *trans* (active) isomer. Consequently, small agonist-induced currents appeared when this solution was allowed to equilibrate with the innervated face of an electroplaque, at

Fig. 1 Bis-Q, a photochromic agonist

$$cis\text{-}bis\text{-}Q \underset{\text{Ultraviolet}}{\overset{\text{Visible}}{\rightleftharpoons}} trans\text{-}bis\text{-}Q$$



−150 mV these initial agonist-induced currents were roughly 2 mA cm^{-2} in the cell of Fig. 2*a*, and 5 mA cm^{-2} in Fig. 2*b* After a voltage jump, relaxations of the agonist-induced current resembled those seen with low concentrations of *trans-bis*-Q There are at least two components. A fast (< 500 μs) phase is kinetically not resolvable and accounts for 10–25% of the relaxation amplitude. This component probably results from instantaneous rectification in the small number of channels opened by *trans-bis*-Q at positive potentials[4] The slower part of the relaxation follows an exponential timecourse, and arises because the number of open receptor channels increases after the voltage jump[4].

We then compared the behaviour of the conductance during voltage-jump relaxations and agonist concentration-jump relaxations induced by light flashes (Fig. 2) An energy of 75 J was discharged through a xenon-filled 'short-arc' flash tube. The light was led through a fibre optic bundle to the pool, roughly a 1-cm cube, bathing the innervated face of the voltage-clamped electroplaque. The fibre bundle filtered out most of the ultraviolet light. Between light flashes the preparation was kept in the dark When the pool contained *cis-bis*-Q, we found with spectral measurements that each flash converted about 2 5% of the *cis* to the *trans* isomer.

Although 'voltage-jump' relaxations occurred with all known agonists[3–6], conductance changes following a light flash were observed only when the solution contained *cis-bis*-Q and not in the presence of agonists, such as suberyldicholine or pure *trans-bis*-Q, which are photostable at the wavelength used. The agonist concentration jump produces an increase of membrane conductance. (We have not yet succeeded in generating ultraviolet flashes strong enough to produce rapid conductance decreases due to *trans*→*cis* isomerisation.) Like the voltage-jump relaxations, the concentration-jump relaxation amplitudes are reversibly diminished about fivefold by (+)-tubocurarine (1 μM).. Thus there seems little doubt that the agonist concentration-jump relaxations reflect a rate-limiting process in the activation of channels controlled by nicotinic acetylcholine receptors

As shown in Fig. 2, the conductance increase is substantially complete within the first few milliseconds following the flash and stable at least until the next episode (4 s later in the experiment of Fig. 2, several minutes later in other experiments) The voltage- and concentration-jump relaxations have similar timecourses at a given membrane potential and temperature. Presumably the rate of *cis*→*trans* isomerisation is at least as fast as the flash, which lasted about 2 ms, after the flash the agonist concentration-jump relaxations followed an exponential timecourse. For the six traces of Fig. 2*a*, measured at 10 °C, the major, exponential component of the voltage-jump relaxation had a time constant of 6.94 ± 0.29 ms (mean ± s e.). The agonist concentration-jump relaxation had a time constant of 6.80 ± 0.28 ms. At 24 °C (Fig. 2*b*), the values were 1.78 ± 0.09 ms for the voltage jump and 1.75 ± 0.14 ms for the agonist concentration jump. On average, the time constants decreased by about 1% after each flash for the first 20 flashes; this observation is not surprising since voltage-jump relaxation times decrease with increasing agonist concentration[3,4]. The time constants vary markedly with temperature, suggesting that channel opening and closing are rate-limited by a chemical, probably macromolecular, process rather than by simple diffusion of newly created agonist molecules toward their site of action.

Several electrophysiological techniques are now available for probing the opening and closing kinetics of acetylcholine receptor channels Thus it is possible to compare (a) the decay of neurally evoked postsynaptic currents and of spontaneous miniature postsynaptic currents[3,4,8–10] (b) the duration of single-channel currents[11]; (c) the power spectrum of ACh-induced conductance fluctuations[5,12,13], (d) voltage-jump relaxations[3–6], and (e) the present agonist concentration-jump relaxations. For the first two experimental procedures, kinetic data are dominated by the closing rates, data from the last three procedures sometimes have comparable contributions from the opening rates as well[4]. The time constants derived from all these experiments agree well in comparable conditions of agonist, concentration, voltage, and temperature. These facts suggest that the opening and closing of the channel are governed either by true first-order processes such as intramolecular isomerisations, or by pseudo-first-order processes such as binding of agonist molecules whose concentration remains essentially constant during the measurement. To distinguish among these possibilities, we require improvements in the agonist concentration-jump technique coupled with similarly rapid measurements of binding and of conformational changes[14].

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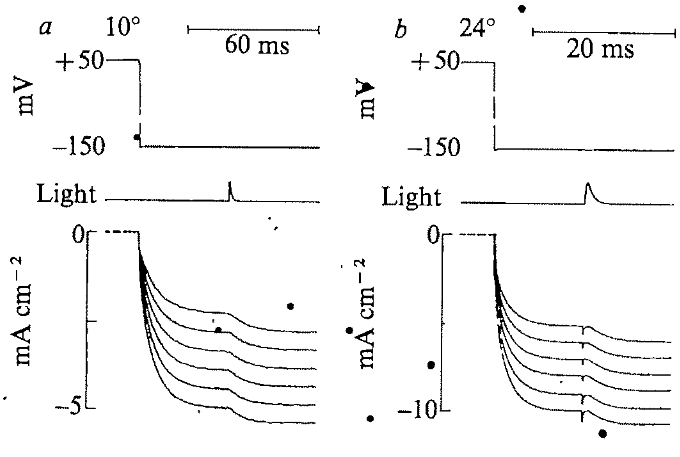
Fig. 2 Voltage-and agonist-jumps in the presence of *bis*-Q (0 4 μM) Two cells at different temperatures, note different timescales for *a* and *b* Each panel shows six superimposed episodes repeated at intervals of 4 s Top trace shows voltage, during each episode, the voltage was jumped from +50 to −150 mV A light flash also occurred later in each episode and was monitored by a phototransistor (middle trace). The episodes shown here included the first six light flashes Bottom traces show agonist-induced currents Separate control trials, measured with the same voltages but without Bis-Q present, gave the leakage and capacitative current alone These currents have been subtracted from the traces shown[4]. Zero current levels were off scale in the original records, they were determined from simultaneous low gain records and are shown as dashed lines Before the first flash about 15% of the *bis*-Q was in the *trans* state, consequently there was an initial level of agonist-induced current Each flash resulted in an increment of *trans-bis*-Q concentration As a result agonist-induced current increased by about 20% of the initial level with each flash



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Release of dopamine *in vivo* from cat substantia nigra

THE central aminergic neurones present some original characteristics. By means of quantitative autoradiographic studies, it has been demonstrated that serotoninergic terminals projecting into the cerebral cortex and the locus coeruleus exhibit very few classical synaptic contacts[1,2]. This has also been seen with cortical noradrenergic terminals[1] It has been suggested that the amines could be released not only in synapses, but also from varicosities devoid of synaptic junctions. they could thus act as neuromodulators at some distance from the release sites. It has also been proposed that dopamine could be released from dendrites of nigrostriatal dopaminergic neurones[3] Indeed, a release of endogenous dopamine (Cuello, personal communication) or of labelled dopamine[4,5] previously taken up in tissues, has been demonstrated using slices of the rat substantia nigra exposed to a high concentration of potassium (30 mM). This process was calcium dependent An indication of possible dendritic release was also obtained indirectly *in vivo* by measuring dopamine metabolites in the rat substantia nigra[6]. Dihydroxyphenylacetic acid and homovanillic acid levels were increased after stimulation of the medial forebrain bundle, which contains axons of various ascending aminergic pathways including the nigrostriatal dopaminergic system Therefore, dopamine could be released not only from nerve terminals in the striatum, but also from dendrites in the substantia nigra. If the latter mechanism was occuring in physiological states, it could have important implications for the understanding of the regulatory processes involved in the control of the activity of the dopaminergic neurones. It thus seemed necessary to demonstrate directly an *in vivo* release of dopamine in the substantia nigra.

Experiments were carried out in halothane-anaesthetised cats. A push-pull cannula was inserted into the substantia nigra (stereotaxic coordinates: $A = 3.5$; $L = 4$, $H = -4$) allowing the superfusion of the structure with artificial cerebro-spinal fluid containing 20–25, μCi per 500 μl L-3, 5-^{3}H-tyrosine (40–50 Ci mmol^{-1}, Radiochemical Centre, Amersham, UK) at a rate of 500 μl per 15 min. ^{3}H-dopamine formed and released extraneuronally, was estimated in serial 15-min superfusate fractions using an isolation procedure as described previously[7] In some experiments the push-pull cannula was introduced into the left caudate nucleus ($A = 16$, $L = 5$; $H = +5$). This experimental approach for the study of dopamine release from nerve terminals has already been used successfully[8]

When the tip of the push–pull cannula was positioned precisely in the substantia nigra, as revealed by postmortem histological control, ^{3}H-dopamine could be easily estimated in the superfusates One hour after the beginning of the experiments, ^{3}H-dopamine release reached a steady-state level (about 20 times the blank value), which persisted during the entire course of the superfusion (Fig 1) As checked by cochromatography, ^{3}H-dopamine was the only labelled catecholamine released (Fig. 2) The quantity of ^{3}H-dopamine spontaneously released into the substantia nigra was 3–4 times lower than that found in comparable experiments carried out in the left caudate nucleus (Table 1).

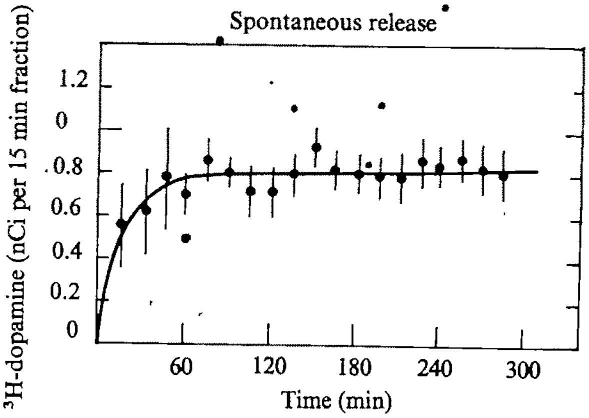

Fig. 1 Spontaneous release of ^{3}H-dopamine from substantia nigra in halothane-anaesthetised cat ^{3}H-dopamine was estimated in serial superfusate fractions (15 min each) collected from a push–pull cannula during the continuous local superfusion of the left substantia nigra with an artificial CSF containing L-3,5-^{3}H-tyrosine (20–25 μCi per 500 μl for 15 min) in halothane-anaesthetised cats Data represent mean ± s e m of results obtained in four experiments

We attempted to demonstrate that various treatments could influence the spontaneous release of endogenously synthesised ^{3}H-dopamine in the substantia nigra When potassium (30 mM) was introduced for 15 min on the CSF, the release of ^{3}H-dopamine was markedly enhanced This stimulating effect was of short duration, the qualities of ^{3}H-dopamine released returned to the steady-state value as early as 15 min after the end of the potassium application (Fig 3A)

(D)-amphetamine and benztropine markedly enhanced the release of ^{3}H-dopamine synthesised from L-3,5-^{3}H-tyrosine in the caudate nucleus[8–10] by stimulating the transmitter release and inhibiting its reuptake It was therefore of interest to determine whether these drugs were exerting similar effects in the substantia nigra, rich in dopaminergic dendrites Both drugs, introduced for 15 min into the superfusing fluid immediately enhanced the transmitter release. The effect was of short duration with (D)-amphetamine, but persisted with benztropine even though the drug had been removed from the artificial CSF (Fig 3*b* and *c*)

These results provide the first direct demonstration of the spontaneous and evoked release of dopamine endogenously synthesised in the substantia nigra in physiological states It can be assumed that the transmitter is released from dendrites, as suggested initially by Bjorklund and Lindvall[3] on the basis of

Table 1 Effects of TTX on ^{3}H-dopamine release from caudate nucleus and substantia nigra of the cat

Structure		n	nCi per 15 min successive fractions 0–15 min.	15–30 min	30–45 min	45–60 min
Caudate nucleus	Control	4	3.14±0.40	3.27±0.28	3.24±0.24	3.21±0.36
	TTX (5×10^{-7}M)	4	2.23±0.17	1.92±0.13*	1.62±0.26*	1.49±0.10*
	% Change.		−29%	−41%	−50%	−54%
Substantia nigra	Control	3	0.84±0.10	0.85±0.11	0.80±0.09	0.82±0.12
	TTX (5×10^{-7}M)	3	1.42±0.16*	1.06±0.14	1.14±0.10*	1.03±0.13
	% Change		+69%	+25%	+43%	+26%

A discrete area of either the caudate nucleus or the substantia nigra of halothane-anaesthetised cats was superfused continuously with an artificial CSF containing L-3,5-^{3}H-tyrosine (20–25 μCi per 500 μl for 15 min) using a push–pull cannula ^{3}H-dopamine was estimated in 15 min successive superfusate fractions TTX (5 × 10^{-7} M) was added for 1 h to the superfusion fluid 180 min after the onset of the superfusion Data represents the mean ± s e m of ^{3}H-dopamine quantities found in the four fractions during TTX application and in corresponding fractions of control animals

* $p < 0.05$ compared with respective control

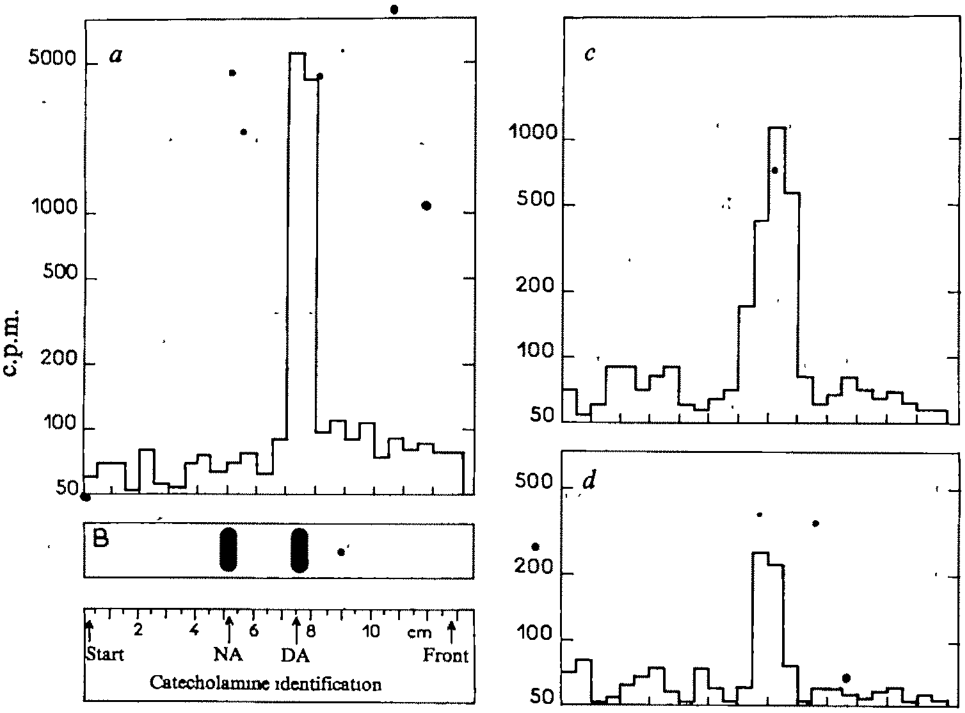

Fig. 2 Identification of the ^{3}H-catecholamine released from the substantia nigra. ^{3}H-catecholamines in superfusates were isolated from ^{3}H-tyrosine and ^{3}H-metabolites by Amberlite ion-exchange chromatography and adsorption on alumina columns. Hydrochloric acid eluates of alumina columns corresponding to successive samples were pooled and submitted to thin-layer cochromatography on cellulose plates using *n*-butanol, acetic acid and water (4.1:2 v/v) as solvent. *a*, ^{3}H-dopamine standard; *b*, spots of the carriers as revealed by ninhydrin; *c*, ^{3}H-dopamine identification in pooled samples collected during the superfusion of the caudate nucleus, *d*, ^{3}H-dopamine identification in pooled samples collected during the superfusion of the substantia nigra. DA, Dopamine, NA, noradrenaline

histochemical studies. Indeed, these authors observed a dopamine-containing varicose dendritic network well organised in the pars reticulata of the rat substantia nigra. Our results confirm and extend the *in vitro* findings of Geffen *et al.*[5] and Cuello and Iversen[4]. ^{3}H-dopamine release was not only enhanced by potassium, but also by drugs affecting the release and/or re-uptake of dopamine in nerve terminals. It seems that dopamine released from dopaminergic dendrites can be partly inactivated by a potent re-uptake process, as observed in nerve terminals. The presence of an active dopamine uptake process in dendrites has already been suggested in biochemical[5], histochemical[3] and autoradiographic studies[11,12]. Exogenous dopamine can be taken up in slices of the rat substantia nigra[4] and visualised in dendrites[3]; labelled dopaminergic dendrites were seen after an intraventricular[11,12] or local injection of ^{3}H-catecholamines[4]. The dopaminergic dendrites contain tyrosine hydroxylase as indicated by a recent

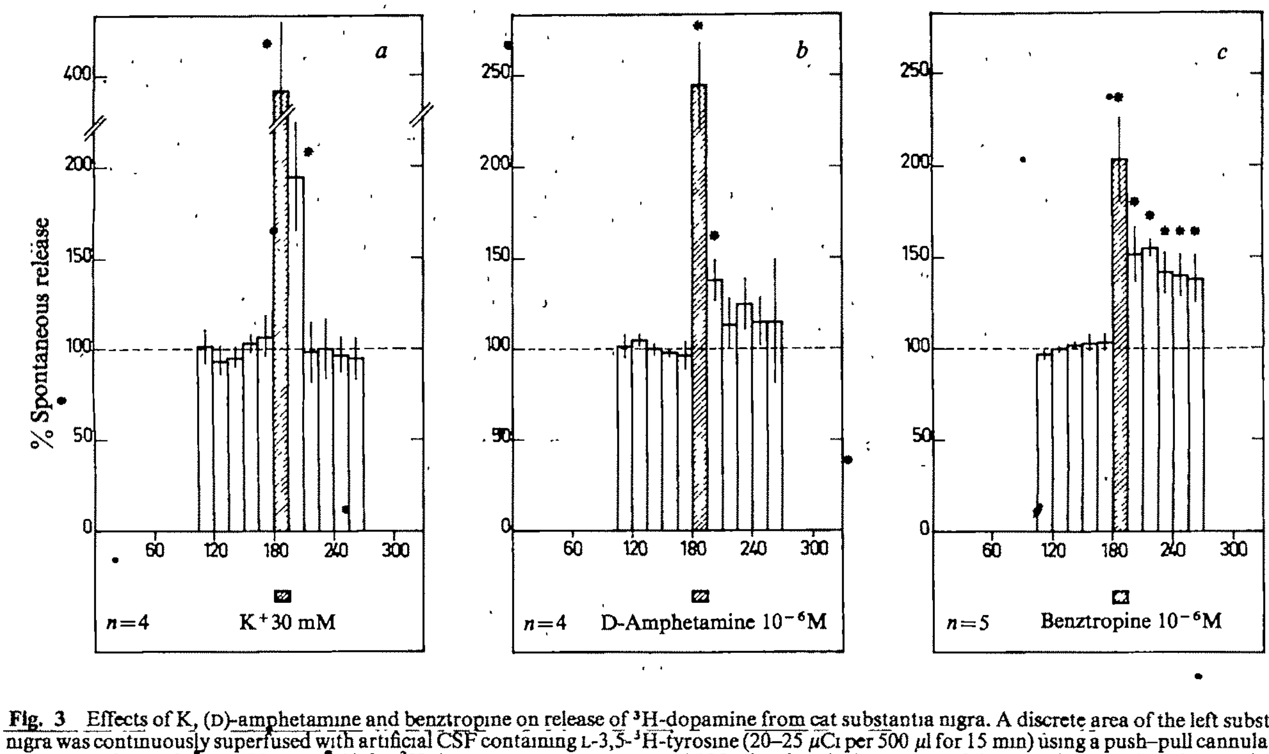

Fig. 3 Effects of K, (D)-amphetamine and benztropine on release of ^{3}H-dopamine from cat substantia nigra. A discrete area of the left substantia nigra was continuously superfused with artificial CSF containing L-3,5-^{3}H-tyrosine (20–25 μCi per 500 μl for 15 min) using a push–pull cannula The collection of superfusates (15 min each) for ^{3}H-dopamine estimation started 105 min after the beginning of the superfusion. Three hours after the onset of the superfusion, K (30 mM), (D)-amphetamine (10^{-6} M) or benztropine (10^{-6} M) was added for 15 min (hatched bars) to the medium introduced into the substantia nigra In each animal, ^{3}H-dopamine in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected before the drug application (105–180 min). Data are mean ± s.e.m. of results obtained with groups of *n* animals *$P<0.05$ when compared with corresponding control values obtained in four animals.

immunohistochemical study[13]. Our results suggest that the local rate of the transmitter synthesis is important and can efficiently supply the release process. In fact, the steady-state level of ^{3}H-dopamine synthesised from L-3,5-^{3}H-tyrosine was reached even faster than in the caudate nucleus[8]

The push–pull cannulae used allowed the superfusion of an area of approximately 2.6 mm^3. Therefore, ^{3}H-dopamine released could originate from the pars reticulata as well as from the pars compacta. It cannot be excluded that ^{3}H-dopamine is partly released from dopaminergic cell bodies or axonal collaterals, or even from hypothetical dopaminergic intrinsic neurones In any case, the release of dopamine at the level of the substantia nigra seems to be somewhat different from that observed in nerve terminals. This could be shown using tetrodotoxin (TTX). By blocking sodium channels, TTX suppressed the nerve impulse flow and subsequently the release of transmitters from nerve terminals. Thus, when TTX was introduced into the CSF superfusing the caudate nucleus, the release of ^{3}H-dopamine was reduced by about 50%, as shown previously[8]. Surprisingly, an opposite effect was observed when TTX was introduced into the substantia nigra In most superfusate fractions, the release of ^{3}H-dopamine was significantly increased (Table 1). This original finding may be interpreted as follows: TTX, by blocking nerve impulse flow in afferences, may suppress tonic inhibitory influences on the activity of dopaminergic neurones. It obviously cannot suppress, however, the release of dopamine from dendrites as seen in nerve terminals, since ^{3}H-dopamine release was increased in the presence of TTX. Therefore, it may be suggested that dopamine release from dendrites is not impaired by blocking sodium channels.

Dopamine released into the substantia nigra seems to have a physiological role. On the basis of microiontophoretic electrophysiological studies, Aghajanian and Bunney[14] postulated that the amine inhibits the activity of dopaminergic cells by acting on dopaminergic autoreceptors This dopamine effect is supported by recent experiments using our cat preparation. Dopamine (10^7 M) introduced into the substantia nigra inhibited the release of newly synthesised ^{3}H-dopamine in the ipsilateral caudate nucleus[15]. This effect was also observed with concentration of (D)-amphetamine and benztropine similar to those used in the present study. Since dendrodendritic contracts have been observed in the substantia nigra[4,16], it can also be speculated that dopamine released from dendrites may affect the activity of neighbouring dopaminergic neurones: lateral inhibition is a process which has been extensively described by neurophysiologists[17] Finally, a dopamine-sensitive adenylate cyclase has been found in the substantia nigra[18–20], particularly in the pars reticulata[20]. Lesion studies have indicated that this adenylate cyclase system is not located in dopaminergic neurones but on descending neuronal afferents[20] which are presumably GABA-ergic or substance P-ergic[4]. The dopamine postsynaptic receptors associated with this dopamine-sensitive adenylate cyclase could be influenced by dopamine released from dopaminergic dendrites It can thus be postulated that local interneuronal feedback processes participate in the control of the dopaminergic neuronal activity Dopamine released *in vitro* from dopaminergic dendrites may modulate nigral afferents at a presynaptic level

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High affinity uptake of glutamate in terminals of corticostriatal axons

GLUTAMIC ACID (glu) may be the transmitter of a large proportion of excitatory synapses in the brain[1], but glu is also important in cell metabolism, and the apparent lack of biochemical 'markers' associated with the role of glu as a synaptic transmitter has until recently hampered the localisation of potential 'glutamergic' neurones. High affinity uptake of glu[2,3] is, however, highly specific[3] and seems to be selectively localised in the excitatory granular cell terminals in the cerebellum[4], and in three systems of excitatory nerve endings in the hippocampal formation, as shown by autoradiography[5] and quantitative measurements[6]. In the conditions used in these studies, glial uptake of glu[7,8] was found not to be quantitatively important. In the hippocampal systems, iontophoretic studies[9] and release experiments[10] strongly suggest that glu, and/or aspartic acid (asp), may be the transmitter. It thus seems that high affinity uptake of glu may be useful as a marker for putative glutamergic and/or aspartergic nerve endings. The question of whether the uptake of [^{3}H]glu, as measured *in vitro,* represents net accumulation or homoexchange[11] is not crucial in the present context. The neostriatum (nucleus caudatus–putamen) receives a large excitatory projection from the neocortex[12,13]. Iontophoretic studies suggest that this could use glu or asp as its transmitter[1,14]. We here demonstrate that high affinity uptake of glu is selectively reduced in the neostriatum after lesions in the neocortex.

Male Wistar rats (250 g) were used. The frontal cortex, between the frontal pole and the frontoparietal suture, was removed down to white matter by suction through a fine needle under microscopic guidance. In sham-operated animals the skull was opened, but no lesion made; unoperated controls were only anaesthetised. After 7 or 14 d the animals were decapitated and frontal slices (about 1 mm) of the brains were prepared in the cold on a Sorvall tissue sectioner. The caudatus–putamen (avoiding the nucleus accumbens) was dissected from the rostral 1–2 slices containing the nucleus to obtain samples receiving cortical afferents predominantly from the lesioned part of the cortex[12]. Histological preparations from other operated animals showed that the caudatus–putamen was not directly damaged by the lesions. The dissected samples were weighed on a Mettler microbalance and homogenised in 50 volumes 0 32 M sucrose solution with 5 mM Na phosphate buffer, *p*H 7.4, in a glass-Teflon homogeniser, to produce suspensions containing nerve ending particles.

High affinity uptakes of glu and γ-aminobutyric acid

Table 1 Selective reduction in high affinity uptake of glu in neostriatum after cortical lesions

Sample	n	Uptake of glu	Uptake of GABA	glu decarboxylase	Choline acetyltransferase	DOPA decarboxylase
L	6	113±12*	30±3	1,970±180	3,590±210	863±72
N	6	193±14†	34±3	1,740±120	3,580±310	870±118
C	7	239±10	32±2	1,800±160	3,480±370	913±53

Results are presented as nmol/min/g protein±s.e.m. of n samples.
L and N, lesioned and contralateral side, respectively, in operated animals; C, right and left sides in sham-operated (2) and unoperated animals (2).
*$P < 0.001$ compared with C; $P < 0.005$ compared with N (Student's t test, both tails).
†$P < 0.025$ compared with C. Other differences were non-significant ($P > 0.2$).

(GABA) were measured by incubating 5 µl homogenate with 500 µl Krebs–phosphate solution at 25 °C for 3 min[6]. The reaction was started by adding 50 µl Krebs solution containing L-[3-^{3}H]glu (New England Nuclear, Boston, Massachusetts, 15.8Ci mmol^{-1}, diluted with unlabelled monosodium glutamate to give a specific radioactivity of 1.14 Ci mmol^{-1}) and [U-^{14}C]-γ-aminobutyric acid (Radiochemical Centre, Amersham, UK, 224 mCi mmol^{-1}) to give final concentrations of 1 µM. Separate studies have shown that GABA and glu do not interact with each other's uptake in such conditions[2,15]. After incubation the samples were diluted with 4 ml ice-cold 0.9 NaCl, collected on Millipore filters (0.45 µm), and rinsed with another two portions of 4 ml NaCl. The filters were eluted in 1.2 ml 10% Triton X-100 and counted with 10 ml Diluene (Packard) in a Packard liquid scintillation spectrometer (model 3380) fitted with an absolute activity analyser (model 544) giving d.p.m. for ^{3}H and ^{14}C separately. Samples incubated at 0 °C, but otherwise treated identically, served as blanks. Experimental and control samples were run together. Uptake of glu and GABA were linear with the amount of striatal tissue up to at least 100 µg wet weight. Protein[16], glutamate decarboxylase[17], choline acteyltransferase[18] and DOPA decarboxylase[19], were determined essentially as described previously.

After lesions of the frontal cortex there was a selective reduction in glu uptake in the nucleus caudatus–putamen, by 41% when the operated side was compared to the contralateral side, or by 53% compared with unlesioned animals (Table 1). The slight reduction on the side contralateral to the lesions compared with unlesioned animals is probably due to the fact that the corticostriatal projection is partly crossed[20]. The reduction in glu uptake on the lesioned side was somewhat larger at 14 d (64% reduction compared with intact controls, 2 animals) than at 7 d survival time (49% reduction, 4 animals), but the results were pooled in Table 1. The lack of change in GABA uptake suggests that the reduction is not due to an unspecific interference with uptake processes. The absence of change in markers for alleged GABAergic (glu decarboxylase, GABA uptake), cholinergic (choline acetyltransferase) and dopaminergic neurones (DOPA decarboxylase) demonstrates that the reduction in glu uptake is not due to a general effect on neurones in the caudatus–putamen. The possibility that the selective reduction in glu uptake could be due to transsynaptic changes—for example, in other excitatory afferents—can not be completely ruled out, but seems rather unlikely.

Since the acidic amino acids, glu and asp, have similar or identical high affinity uptake systems[2,4], and since glu uptake, measured in our conditions, seems to be a useful marker for nerve endings utilising acidic amino acids as transmitters[4,6], our results suggest that the neocortico–neostriatal projection could use glu or asp as its transmitter.

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Benzo[*a*]pyrene 7,8-dihydrodiol is more carcinogenic than benzo[*a*]pyrene in newborn mice

THE pioneering research by James and Elizabeth Miller relating the carcinogenic effects of many chemicals to the metabolism of these compounds to reactive intermediates which bind to critical cellular compoeenents[1,2] led to studies of the nature and chemical structure of these reactive metabolites (ultimate carcinogens) and their metabolic precursors (proximate carcinogens). The environmental carcinogen benzo[*a*]pyrene (BP) is metabolised by microsomal enzymes to arene oxides, phenols and dihydrodiols. Metabolic activation of the BP metabolite (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP 7,8-dihydrodiol) by liver microsomes resulted in more binding to DNA than did the metabolic activation of BP (ref 3). The reactive metabolite involved in the binding to DNA was a *trans*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro BP (BP 7,8-diol-9,10-epoxide) (ref. 4). Both stereoisomers of this diol epoxide, which are enzymatically formed from BP 7,8-dihydrodiol (refs 5–8), were highly mutagenic to bacterial and mammalian cells[9-15] suggesting that a BP 7,8-diol-9,10-epoxide might be an ultimate carcinogenic metabolite of BP. We report here that administration of BP 7,8-dihydrodiol to newborn mice caused more malignant lymphomas and pulmonary adenomas than did administration of benzo[a]pyrene. This is the first demonstration of a metabolite of a polycyclic aromatic hydrocarbon that is more carcinogenic than the parent compound

Table 1 Carcinogenic activities of BP, BP 7,8-dihydrodiol and diol epoxide-*2* in newborn mice

Treatment	Total dose (nmol)	Sex	No of survivors at 25 d	Age 4–17 weeks			Age 18–24 weeks			
				No. of animals autopsied	No of animals with		No. of animals autopsied	No of animals with		
					malignant lymphoma	lung adenomas		malignant lymphoma	lung adenomas	other tumours
Control	0	♂	34	—	—	—	32	0	2	
		♀	17	—	—	—	16	0	0	
		Total	51	—	—	—	48	0	2	
BP	1400	♂	26	—	—	—	24	0	18	*
		♀	24	1	0	1	20	0	19	
		Total	50	1	0	1	44	0	37	
BP 7,8-dihydrodiol	1400	♂	24	9	5	3	12	8	11	†
		♀	40	17	12	5	12	10	11	‡
		Total	64	26	17	8	24	18	22	
Diol epoxide-*2*	28	♂	17	—	—	—	17	0	11	
		♀	20	—	—	—	20	0	19	
		Total	37	—	—	—	37	0	30	

*Two mice with fibroma of the skin

†Two mice with skin papillomas.

‡One mouse with fibroma of the skin

Swiss Webster mice [BLU.Ha(ICR)] were injected i p with BP and BP derivatives One-seventh of the indicated total dose of hydrocarbon was given on d 1 of life, two-sevenths on d 8 and four-sevenths on d 15. Control animals were given the vehicle, DMSO. The mice were weaned when 25 d old. The data were obtained from the survivors of experiments 1 and 2 described in Fig. 1 Animals more than 25 d old which died during the course of the study and animals killed at the end of the study (when the animals were 21–24 weeks old) were subjected to histological examination A few animals which died after weaning were cannibalised and could not be examined.

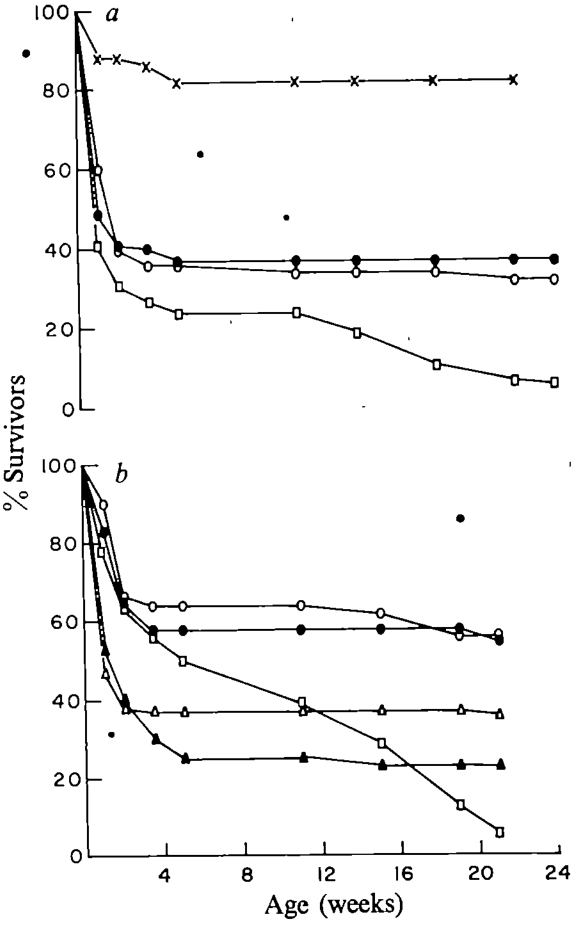

Fig. 1 Toxicity of benzo [*a*]pyrene derivatives in newborn mice Swiss–Webster mice [BLU·Ha (ICR)] were injected i.p. with BP or BP derivatives One-seventh of the total dose of each compound was given on d 1 of life, two-sevenths on d 8 and four-sevenths on d 15. In experiment 1 (*a*), 50 animals were each injected with 1,400 nmol of BP (○), 70 animals with 1,400 nmol of BP 7,8-dihydrodiol (□), 70 animals with the vehicle (DMSO) (●) and 50 animals were untreated controls (×) In experiment 2 (*b*), 50 animals were treated with the above dose of BP, 80 animals received the above dose of BP 7,8-dihydrodiol and 40 animals were given DMSO Diol epoxide-*1* (28 nmol) (▲) was injected into 40 animals, and diol epoxide-*2* (28 nmol) (△) was injected into 100 animals The mice were weaned when 25 d old.

One of the stereoisomeric BP 7,8-diol-9,10-epoxides ((±)-*trans*-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro BP; diol epoxide-*2*; also termed diol epoxide I (ref. 11) or anti-isomer (ref. 16)) produced about the same number of pulmonary adenomas as a 50-fold higher dose of BP.

BP 7,8-dihydrodiol, diol epoxide-*1* [(±)-*trans*-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro BP; also termed diol epoxide II (ref 11) or syn isomer (ref. 16)] and diol epoxide-*2* were synthesised as previously described[17,18], and all chemicals were of analytical-grade purity Pregnant Swiss–Webster BLU:Ha (ICR) mice (Blue Spruce Farms) delivered their babies within 2–5 d after arrival in our laboratory. The healthiest-looking newborns were selected, and each mother was left with 10 babies. BP and BP 7,8-dihydrodiol were dissolved in anhydrous dimethylsulphoxide (DMSO) (ref. 10) at a final concentration of 200 nmol per 5 μl. The compounds were injected intraperitoneally (i.p.) as follows: 200 nmol on the first day of life, 400 nmol 1 week later, and 800 nmol at 15 d of age. Control animals were given 5, 10 and 20 μl of DMSO using the above schedule. The newborn mouse is very sensitive to i.p. injections, and many animals died within 2 weeks following injection with the control vehicle or with the various compounds on days 1, 8 and 15 of life. The animals were weaned at 25 d of age. Large differences in survival among animals treated with DMSO, BP or BP 7,8-dihydrodiol did not occur until after the animals were 25 d old (Fig. 1). The experiments were ended 21–24 weeks after their initiation. After the initial deaths in all groups during the first 2 weeks of age, most of the remaining BP 7,8-dihydrodiol-treated animals died by 24 weeks of age, whereas most of the BP- and DMSO-treated animals that survived until weaning were still alive. Complete histopathological examination was performed on most animals (see Table 1 legend). The examination included routine study of the hematopoietic tissues and counting of macroscopic pulmonary adenomas. Seventy per cent of the BP 7,8-dihydrodiol-treated animals thus examined developed malignant lymphoma (leukaemia), which was apparently a major cause of death of these animals (Table 1). Almost all cases of malignant lymphoma were generalised with involvement of the thymus, spleen, bone marrow, lymph nodes, liver and other organs. Of special interest was the very short latency period for the development of malignant lymphoma. The first diagnosis of malignant lymphoma was made in an animal treated with BP 7,8-dihydrodiol, which died at 5 weeks of age. No cases of malignant lymphoma were recorded among the BP- and DMSO-treated animals. BP 7,8-dihydrodiol also induced pulmonary adenomas. Although the first pulmonary adenoma was found at an early age (14 weeks), induction of this type of tumour was slower than that of the malignant lymphomas. Among the BP 7,8-

dihydrodiol-treated animals which died and were autopsied between 4 and 17 weeks of age, 31% had lung adenomas, and 65% had malignant lymphomas. Among the BP 7,8-dihydrodiol-treated animals which died or were killed at the age of 18–24 weeks, 92% had lung adenomas and 75% had malignant lymphomas. The BP-treated animals also developed lung adenomas, but the number of pulmonary adenomas per animal was much lower when compared to BP 7,8-dihydrodiol-treated animals (Table 2). Only 4% of the DMSO-treated animals developed pulmonary adenomas. Three animals treated with BP 7,8-dihydrodiol developed a total of two skin papillomas and one skin fibroma. Fibroma of the skin was also found in two of the BP-treated animals. There were no significant differences in death rate or tumour incidence between male and female animals.

Table 2 Pulmonary adenomas in newborn mice treated with BP, BP 7,8-dihydrodiol and diol epoxide-*2*

Treatment	Total dose (nmol)	Sex	No. of animals	Adenomas/animal (mean ± s.e.)
Control	0	♂	32	<0.1
		♀	16	0
		Total	48	<0.1
BP	1400	♂	24	5.1±1.6
		♀	20	7.7±1.3
		Total	44	6.3±1.0
BP 7,8-dihydrodiol	1400	♂	8	77±16
		♀	6	72±27
		Total	14	75±14
Diol epoxide-*2*	28	♂	17	2.8±1.0
		♀	20	6.8±1.1
		Total	37	4.9±0.8

Swiss–Webster mice [BLU.Ha (ICR)] were injected with BP and BP derivatives (Fig 1, Table 1) Animals that were killed at the end of the study, at 21–24 weeks of age, were used for determination of the number of adenomas per lung.

In a preliminary experiment, newborn mice were injected separately with each of the two diastereomers of BP 7,8-diol-9,10-epoxide (diol epoxide-*1* and diol epoxide-*2*). These compounds were very toxic, and 80–90% of the animals died within 48 h after a single injection of 200 nmol of diol epoxide-*1* or -*2*. A reasonable survival of animals was achieved only after decreasing the dose of these diol epoxides to 2% of the dose of BP or BP 7,8-dihydrodiol. Four nmol of diol epoxide-*1* or -*2* was given on d 1 of life, 8 nmol on d 8 and 16 nmol on d 15. However, this dose of diol epoxide was still more toxic to the animals than a 50-fold higher dose of BP or BP 7,8-dihydrodiol (Fig. 1, experiment 2). The experiment was ended when the mice were 21 weeks old, and lung adenomas were found in 81% of the animals treated with diol epoxide-*2* (Table 1). For diol epoxide-*2*-treated animals which had survived until weaning, about the same incidence of pulmonary adenomas had occurred as with a 50-fold higher dose of BP (Table 2) Diol epoxide-*2* did not induce malignant lymphomas in the newborn mice Diol epoxide-*1* was more toxic than diol epoxide-*2* in the newborn mice (Fig 1 and unpublished observations), and there were not enough survivors available for determining the carcinogenicity of diol epoxide-*1*. Additional studies are now in progress to determine the comparative carcinogenicity of these two diol epoxides of BP.

Studies on the carcinogenicity of BP metabolites on mouse skin showed that BP 7,8-oxide was carcinogenic[19] and that BP 7,8-dihydrodiol was a more potent carcinogen than BP 7,8-oxide (ref 20). H_4-BP 7,8-epoxide, however, which is related to BP 7,8-oxide but lacks the double bond in the 9,10-positions of the molecule, did not show any carcinogenic effect, suggesting that a BP 7,8-diol-9,10-epoxide might indeed be an ultimate carcinogenic metabolite of BP Since these earlier studies with BP 7,8-dihydrodiol and BP were performed with doses which were at the top of the dose–response curves, no conclusion was reached as to whether BP 7,8-dihydrodiol was more carcinogenic than BP. Our results here indicate that BP 7,8-dihydrodiol is a more potent carcinogen than BP when injected into newborn mice and is therefore a proximate carcinogenic metabolite of BP. Diol epoxide-*2*, a very potent mutagen derived from BP[10–12], has the same carcinogenicity as BP at a 50-fold lower dose in newborn mice. The extremely high carcinogenic activity of diol epoxide-*2* suggests that it is an ultimate carcinogenic metabolite of BP.

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matters arising

Lunar and meteoritic palaeomagnetism: common origin contested

BANERJEE and Mellema[1, 2] (BM) have suggested that both the lunar crust and the primitive carbonaceous chondrites (CC) have been magnetised simultaneously by strong ($\lesssim 1$ Oe) magnetic fields associated with an early T-Tauri active phase of the Sun. Here I argue that this conclusion is untenable. Their basic premises are, first, similar ages of, and, second, comparable palaeofield strengths for, lunar rocks and CC.

The CC and their constituents are among the oldest solar system objects dated thus far[3-7] ($\sim 4.6 \times 10^9$ yr). Sensitive ^{129}I chronometry revealed sharp isochronism for CC magnetite formation (within 2×10^6 yr) and indicated rapid aggregation and brief metamorphism, lasting at most 1 Myr (ref. 5). An upper limit on the duration of a precompaction irradiation episode of CC component grains (by solar wind and solar flares) was recently estimated at < 4.5 Myr, but typically $\lesssim 1$ Myr, at nominal heliocentric distances of 10 AU (ref. 8). These and the enrichment of solar-wind implanted gases in CC magnetite[9] strongly support my earlier suggestion that an enhanced early solar wind, convecting strong magnetic fields[10], could account well for several observed magnetic features of CC[11]. Moreover, recent results on the electrical properties of CC[12] seem to link their magnetic and thermal history satisfactorily in the framework of solar-wind induction heating of asteroids with carbonaceous surfaces, which at present dominate by number in the main-belt[13]. Observations of young active stars[14] and calculations of 'T-Tauri lifetimes' for $\sim 1 M_\odot$ stars[15,16] show that an epoch of intense mass loss for an evolving Sun could have lasted 1–10 Myr. This is compatible with the above chronology of CC, but raises a major difficulty for BM in the case of lunar rocks.

First, the early chronology of lunar crust differentiation cannot be similarly established due to an early intense bombardment, which lasted up to $\sim 3.9 \times 10^9$ yr ago and led to its extensive brecciation and metamorphism[17]. Second, since BM assume implicitly that lunar rocks carry a thermoremanence (TRM) acquired at cooling in an external magnetic field, it is reasonable to date it by the crystallisation ages of lunar basalts and breccias, which range from ~ 3.3 to $\sim 4 \times 10^9$ yr (ref. 17). This requires an excessively long (0.6–1.3×10^9 yr) active phase for maintenance of strong fields. Also, it is unlikely that an older magnetisation (4.6×10^9 yr) has survived the violent impact metamorphism of the ancient lunar crust[17]. Furthermore, a strong case was recently made for excluding any external source field for globally magnetising the ancient lunar crust[18, 19], so that the BM suggestion is weakened on both theoretical and observational grounds.

Table 1 Palaeofield intensities from carbonaceous chondrites

Meteorite (type, subtype)	Palaeofield (Oe)	pTRM (T °C, H Oe)	NRM fraction matched	NRM type	Ref.
Orgueil (I, C1)	0.67	(120, 0.5)	$T_B < 73$ °C	TRM	21
	0.02	(250–120, 0.5)	$120° < T_B < 250$ °C	CRM	21
Ivuna (I, Cl)	0.5	—	Whole coercivity spectrum	(CRM+ DRM)	11
Murray (II, C2)	0.7 (0.33–1.35)	(250, 0.5)	$H_{AF} > 100$ Oe	Non-thermal	11
Murchison (II, C2)	0.4	(250, 0.5)	$100 < H_{AF} < 300$ Oe	Non-thermal	11
	< 3		$H_{AF} > 300$ Oe	pTRM ?	
	0.18 (0.17–0.19)	(90, 0.5)	$T_B < 90$ °C	TRM or CRM	21
	0.01–0.02	(90–250, 0.5)	$90° < T_B < 150$ °C		
Renazzo (II, C2)	2.3	(250, 0.5)	All	pTRM or TCRM	11
Allende (III–V, C4)	0.95	(150, 0.5)	$H_{AF} < 500$ Oe	Non-thermal	11
	0.25		$H_{AF} > 500$ Oe	pTRM	
	0.15	(350, 0.05)	$400 < H_{AF} < 600$	Non-thermal	
	1.09(0.96–1.23)	(110–130, 0.5)	$20° T_B < 130$°C	TRM	21
	1.1(0.93–1.3)	(150, 0.59)	$30 < T_B < 150$°C	TRM, TCRM	22

H_{AF}<peak alternating field value which gives microcoercivity of remanence; T_B, blocking temperature; (p) TRM, (partial) thermoremanent magnetisation; TCRM, thermochemical remanence; CRM, chemical remanence; DRM, depositional remanence.

The claim that comparable paleaofield estimates of $\lesssim 1$ Oe for lunar rocks and CC should be taken at face value can also be challenged. Before discussing palaeointensities we must be concerned with the presence of a demonstrably ancient NRM component. CC evidence looks quite convincing[11,20-22], but it is still doubtful that any lunar rocks have preserved an indigenous palaeoremanence[23, 24]. The mechanism by which this NRM was imprinted is also at issue: the CC are compositionally the most primitive and thermally the least altered extraterrestrial samples known. Their NRM is admittedly a low temperature magnetisation, although detailed interpretations differ[11,21,22] (Table 1). In contrast, most lunar rocks for which palaeointensities have been obtained are thermally and mechanically modified by impacts.

It is even doubtful whether lunar rocks are capable of acquiring TRM in the conventional sense[26, 27].

In this light, the fact that entirely different experimental techniques have yielded similar values for the ancient field strength (~1 Oe) in several CC[11, 20] (Table 1) inspires confidence in their validity. In contrast, the lunar palaeointensity determinations are notoriously controversial[23,25–27]. Some of the new techniques used (refs 25, 28, 29) are not yet established even for terrestrial rocks, whose magnetic mineralogy is more familiar and better understood. Banerjee and coworkers fuelled the debate with 'reliable' palaeofield estimates of ~0.01 Oe for three Apollo 15 basalts[28, 29], including one (15535) which they had earlier shown to contain no useful remanence at all[30,31]. Their later work on an Apollo 17 foliated breccia boulder produced no less than three distinct sets of paleointensities (0.18–0.74 Oe)[32, 33], differing significantly both within and between adjacent foliation layers. These and divergent directions of 'stable' palaeoremanence forced the conclusion that the lunar magnetising field must have changed considerably both its direction and its intensity during the assembly of this 2-m boulder 4.0 × 10⁹ yr ago. It seems more plausible to argue that the magnetic data are not internally consistent, or to ascribe the magnetic behaviour of breccias to textural effects of compaction shock[24].

Another corollary to the BM hypothesis is that similar palaeofield strengths imply a common source for lunar rocks and CC. Such reasoning could lead to the fallacious conclusion that the oldest (3.85 × 10⁹ yr) terrestrial rocks were also magnetised by extended solar wind fields. Within the BM context, comparable magnetic field strengths imply that the Moon and the carbonaceous meteorites condensed and accreted at the same heliocentric distance, in contradiction to most current chemical nebular models[34]. If the CC have originated in the asteroid belt[13] (3–5 AU), whereas the Moon accreted near 1 AU, the present radial dependence of the solar wind magnetic fields would require at least 3–5 Oe for a tightly wound spiral configuration, but up to 10–25 Oe for radial flow. These values are high even by the currently inflated lunar-magnetic yardstick.

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BANERJEE REPLIES—There was one major point that we had wanted to make in our original communication[1]. Before, and even since the publication of our note, there have been no lunar palaeointensity experiments in which, first, a direct, thermal method had been used on three sub-samples from one rock and, second, magnetic monitoring by Anyhsteretic Remanent Magnetization (ARM had been carried out on the samples before and after the heating. In spite of the well-known magnetic variability of lunar samples, as underlined by Dr Brecher[2], what was amazing to us was that our three samples were magnetised homogeneously enough to yield an average palaeointensity of 0.4 Oe with a s.d. of about 40%. In contrast, in the lunar palaeointensity measurements, the highest reliability that one usually hopes to achieve is the order of magnitude of the field!

Our second point was that if the palaeointensity was indeed of the order of 0.4 Oe at about 4.0 × 10⁹ yr (the average radiometric age of the rock), and if the field was internally generated from the Moon, the Sonnett and Runcorn[3] dynamo in a small, early lunar iron core could be the only plausible internal source. We then drew attention to the similarity of the size of this field to that postulated earlier for carbonaceous chondrites (age ~ 4.4 × 10⁹ yr) by us[4], and by Dr Brecher[6]. I agree wholeheartedly with her that "the fact that entirely different experimental techniques have yielded similar values for the ancient field strength (~1 Oe) in several CC (carbonaceous chondrites) inspires confidence in their validity. In contrast, the lunar palaeointensity determinations are notoriously controversial". But, we have done our best in searching for better methods, used multiple subsamples and published the details of our technique[6]. Our interpretation, and that of any other groups of scientists, must necessarily be a function of our knowledge at the time of writing. None of the references quoted by Brecher can convince anyone that "it seems more plausible to argue that the magnetic data (that is, the data of refs 1 and 6) are not internally consistent", specifically when such a search for internal consistency of palaeointensities has not been undertaken.

Our hypothesis that both the meteorites and the ancient lunar samples could have been magnetised by external solar fields is admitted by the fact that the upper limit T-Tauri phase solar wind pressure would correspond to a magnetic pressure of the order of a few Oe at 1 AU. But, as we pointed out, whether this was actually so cannot be proved without more extensive and reliable data on both meteorites and lunar samples. We had also suggested that the apparently large (0.4 Oe) field could be the result of a smaller ambient solar field, magnetohydrodynamically amplified by plasma dynamos operating on the lunar crust during the intense collisioning stage, at and before 4.0 × 10⁹ yr. I am sure there are others who could think up more alternative explanations. The purpose of our communication was to alert other workers about the existence of our data and to encourage them to think of plausible models.

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Digynic triploidy after superovulation

DIGYNIC triploidy after superovulation in mice reported by Takagi and Sasaki[1] may be closely related to the aging of eggs at fertilisation rather than superovulation through the administration of exogenous gonadotrophins. It is well known that the fertile life of eggs after ovulation is about 10 h and that ageing of eggs results in a decrease of fertilisation rate, an increased incidence of abnormal fertilisation and subsequent degeneration of embryos. Suppression of the second polar body formation resulting in digynic fertilisation occurs when eggs age in the hamster[3], mouse[3] and other species[4]. Since the ovulation of a large number of eggs is not expected to occur simultaneously and the time of ovulation for each individual egg would be more spread out during superovulation than after normal ovulation, the chances of delayed fertilisation and digynic triploidy would be higher after superovulation than normal ovulation. Most of the fertilised eggs from superovulated rabbits, however, can develop into normal young after transferring them to several recipient rabbits[5].

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Nitrate reductase as predictive test of crop yield

THE report of Johnson, Whittington and Blackwood[1] on using nitrate reductase as a predictive test of crop yield is probably valid but there are several problems with both the *in vitro* and *in vivo* assay they used which deserve attention.

The maximal *in vitro* activity shown in Fig. 1 of ref. 1 is about 0.1 μmol g^{-1} h^{-1}, a value considerably lower than the activities (~10–12 μmol g^{-1} h^{-1}) commonly observed in leaves of wheat[2-4]. The disparity is so large as to cause concern about the assay procedure. Although the latter activities were obtained without casein in the extraction medium it is conceivable that the inordinately low activities obtained by Johnson *et al.* were partially due to failure to protect nitrate reductase from a constitutive serine protease which rapidly degrades nitrate reductase *in vitro*[2,5,6]. In *Escherichia coli* a serine protease acts to remove nitrate reductase from membranes[7] and in higher plants this may be a reason why its half life is in the order of 2–4.2 h (refs 8, 9). Incorporation of casein or phenylmethylsulphonylfluoride (PMSF) into the extraction media[5,6] obviates this protease activity and yields activities approximately 100 times[10,11] those reported by Johnson *et al.* Therefore, if *in vitro* nitrate reductase activity is to be used as a predictive assay, it should be assayed as described above to avoid complications resulting from differential degradation during extraction.

The problem with the *in vivo* assay of nitrate reductase is also serious. This assay may or may not be proportional to the *in vitro* activity[12,13], but typically represents only about 20% of the *in vitro* activity[13]. Also, the electron donor for the *in vivo* assay has never been identified, nor is it clear that the electron donor *in situ* is identical to that which serves in the conditions employed in the *in vivo* assay. Therefore, it seems unwise to use this assay to estimate any parameter associated with nitrate assimilation unit it is established exactly what the *in vivo* assay is measuring.

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JOHNSON *et al.* REPLY—While a number of interesting points are raised by Butz, it is difficult to see why his criticisms are directed specifically at our work. In any study where enzyme activities are measured either *in vitro* or by the so-called *in vivo* assays it is difficult to state categorically whether or not the measured rates reflect accurately the true activities of the enzymes within the cells. The essential point surely is that our experiments[1] and in the many experiments from Hageman's group in Illinois[2,3], a correlation has been shown between nitrate reductase activity as measured, and the yield of a crop. For such a correlation to be valid or useful the precise biochemical basis is not necessarily important. In this case, however, the correlation does provide valuable evidence that the enzyme activity measured is related to the process of nitrogen metabolism.

Some of the points raised by Butz do require some further comment. First, the references to the work of Schrader *et al.*[4,5] are misleading. In their investigations the improvement in nitrate reductase activity obtained using casein was strongly dependent on the type and age of the tissue employed. With the youngest leaf tissue (similar to that used in our study) the improvement was only 10% compared with the 100-fold quoted. This latter value was obtained only with old tissue of one particular variety of maize and cannot be considered in any way typical. In our investigation some of the *in vitro* wheat nitrate reductase assays were duplicated with *in vivo* assays. These gave substantially similar results. Casein is nevertheless a useful protector of nitrate reductase *in vitro*, although it is by no means the most effective one in all tissues.

Second, Butz speculates that the protease which attacks nitrate reductase does so by removing the enzyme from membranes. This seems unlikely as neither in *E. coli* nor, judged by the majority of the available evidence[6], in higher plants is assimilatory nitrate reductase membrane bound.

Third, in view of the problems known to be associated with the *in vitro* assay of nitrate reductase, of which those referred to by Butz are by no means all, it seems to us unwise to criticise an *in vivo* merely because it sometimes produces results differing from those obtained with an *in vitro* procedure. The fact that the electron donor is not known does not seem important in this context. Recent reports of differences between results obtained using the two procedures may have obscured the fact that in the majority of cases very similar results are obtained with both methods. For example, Hageman's group[3] showed a correlation of 0.99 between *in vitro* and *in vivo* assays of nitrate reductase in several varieties of wheat. Furthermore, a good correlation was obtained between nitrate reductase activity and nitrogen assimilation, thus confirming our observations.

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Control of feline leukaemia virus

HARDY *et al.*[1] report the success of a programme for preventing spread of feline leukaemia virus (FeLV) among pet cats by screening for FeLV viraemic individuals, removal of carriers and quarantine of contacts. Although evidence that the programme was of some success seems strong, the study's methodology deserves comment. Major problems concern the comparisons between 'treated' and 'untreated' groups.

(1) The groups were 'self-selected' by owners. Though it is probable that pet owners' responses to such a programme reflect different approaches to the care of pets, we are given no information on the cluster-size distribution, location, management, sex or age structures of the groups—though each such variable has major implications for the spread of an infectious agent.

(2) The only information given as to the previous status of the groups is that the treated group had a lower initial prevalence rate of FeLV carriers than did the untreated control (0.224–0.312). This indicates that the treated group was at a lower risk of infection even before the programme and thus it is improper to attribute subsequent differences in incidence rates to the programme alone.

(3) The report of a 0.193 incidence rate in the untreated group over three months is surprising, as it implies an annual incidence rate of $1.0-(1.0-0.193)^4$ or 0.576, which is far higher than the prevalence rate in either group—though the authors claim that most viraemic cats remain viraemic for life, they report a low natural mortality rate for viraemic individuals and they make no reference to reversions to virus negative among the untreated group cats.

(4) The programme entailed both removal of viraemic individuals and also quarantine of contacts. As neither action was imposed upon the untreated group it is improper to attribute the lowered incidence rate to removal of FeLV carriers alone.

(5) Mortality 'rates' are given without reference to time period or to background mortality among 'uninfected' cats. Without such references they are uninterpretable.

(6) The authors' conclusion that infection in most cats occurred between 18 and 24 months of age does not follow logically from their data on median ages—and, if it did, it would emphasise the necessity to adjust for age when comparing incidence and mortality rates. No such adjustments have been made.

The implications of Hardy *et al.*'s study are important. Though some of the problems mentioned here may have arisen due to the demands of brevity in publication, it should be stressed that such studies deserve a careful methodology. This is especially true in the context of current interest in the epidemiological implications of oncornaviruses.

P. E. M. FINE

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[1] Hardy, W.D. *et al.* Nature 263, 326–328 (1976).

HARDY AND MCCLELLAND REPLY—We agree with Dr Fine that a 42-fold decrease in feline leukaemia virus infection (FeLV) in the households which implemented our FeLV test and removal programme is evidence that our programme was successful[1]. Our study was not a strict epidemiological survey, indeed, an epidemiological study of lymphosarcoma in cats failed to detect the contagious nature of the disease[2]. But, we would like to answer Dr Fine point by point.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

(1) All the owners were given the same advice regardless of whether or not they chose to have their infected cats removed. The management of the households was the same for both groups and the average number of cats per household (Fine definition of cluster) in those households in which the cats were removed was 16.6 cats per household while in the other group it was 16.5 cats per household (Table 2, ref. 1). Households in both groups were located throughout the USA. The two groups are thus quite comparable.

(2) Although it is true that the initial FeLV infection rate of the two groups was different, the difference in the rates of FeLV infection between the two household groups after our programme had been implemented was much greater and was statistically highly significant ($P<0.001$).

(3) Dr Fine should not assume that the FeLV infection rate of 19.3% in the first 3 months will be perpetuated. The infection rate decreases because, as we reported, approximately 41% of the exposed cats in a household develop protective FeLV neutralising antibody titres though they never test positive for FeLV. We reported previously that 33% of healthy exposed cats became FeLV infected[3]. This figure was the result of a single sampling of many households in which the cats had been exposed to FeLV for varying periods of time. In some households, 100% of the cats were infected, while in others only 10% were infected, which shows that the FeLV infection rate can vary greatly. In contrast to Dr Fine we do not think that our observed mortality rate of 52% for secondarily infected cats and 8.5% for first test FeLV uninfected cats is low. With regard to his other points in this area; most cats do remain viraemic for life and reversions happened only rarely among the cats in this study.

(4) Both groups of households were quarantined as referred to in refs 13 and 16.

(5) The observation period for the study was 2 y, as mentioned in the paper[1].

(6) The median age of cats which became infected with FeLV was simply our observation on 49 cats—we did not make any conclusions about age susceptibility of pet cats in the general population.

In conclusion, we would like to add that removal of the carrier host is a common method for controlling and preventing the spread of many infectious veterinary diseases when vaccination is not available. For instance, bovine tuberculosis, brucellosis, foot and mouth disease, hog cholera, African swine fever, vesicular exanthema, rinderpest and glanders are controlled by test and removal programmes. Thus, the concept of preventing the spread of the contagious feline leukaemia virus by a similar programme should not, we think, be difficult to accept.

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[1] Hardy, W D *et al. Nature* 263, 326–328 (1976).
[2] Schneider, R *Int J Cancer* 10, 338–344 (1972).
[3] Hardy, W. D., Jr, Old, L. J, Hess, P. W, Essex, M. & Cotter, S. M. *Nature* 244, 266–269 (1973).

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HYPERTENSION

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edited by W. DE JONG, A. P. PROVOOST *and* A. P. SHAPIRO

PROGRESS IN BRAIN RESEARCH, Vol. 47

1977. about 380 pages.
US $ 57.95/Dfl. 142.00. ISBN 0-444-41534-3

This book contains contributions by over forty international authorities on the control of arterial blood pressure by the brain. These centre on three main areas: 1) integration of neuroanatomy, neurophysiology and neurotransmission of the central nervous system in relation to cardiovascular control and emotional behavior in man and animal; 2) brain damage, i.e. infarction, blood flow disturbances, stroke, etc, resulting from hypertension and vice versa; 3) evaluation of brain mechanisms in drug and non-pharmacological treatment of hypertension, including bio feedback, relaxation techniques and the role of the social environment.

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by W. H. BIRKENHÄGER *and* M. A. D. H. SCHALEKAMP

1976. xviii + 182 pages.
US $26.75/Dfl. 65.00. ISBN 0-444-41452-5

from the introduction

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Circle No. 08 on Reader Enquiry Form.

Entering the genetic arena

William J. Schull

Who Do You Think You Are? Man or Superman. The Genetic Controversy. By Oliver Gillie Pp. 255. (Hart-David, MacGibbon: London, 1976.) £4.95.

OF LATE, genetics, and particularly human genetics, has found itself increasingly involved in controversial social issues; these include the roles of heredity and environment in the origin of the apparent differences in performance on intelligence tests, and the relationship, if any, of an extra Y chromosome to criminality. The scientific discussion of these issues has been characterised by more intellectual arrogance and humbug than seems generally to prevail. I had hoped that this little volume might redress these excesses in a trenchant way. It does not. *Who do you think you are?* has a catchy title, a few provocative thoughts, but is generally disappointing. We are confronted with clichés, and an overly pejorative division of the world into hereditarians and environmentalists. The attributes imputed to these groups would be worthy of a medieval scholastic obliged to account for the presence of evil in a world produced by a deity intrinsically good. What of value can possibly come from further polarisation of the attitudes toward these problems? These are strong, possibly intemperate remarks to some. Let me document them.

We find on p14 such simple clichés about political beliefs as, " 'Liberal' or 'radical' scientists tend to be 'environmentalists' and 'conservative' scientists tend to be 'hereditarians' who believe that heredity is of preeminent importance in deciding character. This is exactly what we might expect when we reflect that it is conservatives who always have the greatest interest in property and titles—that is, the greatest vested interest in non-biological inheritance." Or on pp109–110 we are treated to an analysis of Johannes Lange's personal prejudices and how the latter coloured not only the data on criminality he collected but his interpretations of these data as well. These accusations may indeed be true, but the cast of the argument seems to indict all "hereditarians" but exonerate "environmentalists" of this not so admirable human failing. One wonders whose prejudices are showing here. The larger issue, or so it seems to me, is not whether Lange or others exhibit prejudices but what is the sequence of events or experiences, cultural or biological, which gives rise to them. Self-serving irrelevancies are everywhere. The late Trofim Lysenko is described as being "from a simple peasant background" (p24); arguments which the author favours are prefaced with remarks such as "few serious scientists now dispute' (p128)—the implication is clear. Darlington's *Genetics and Man* doubtlessly deserves the criticisms which Gillie levels, but who other than he has ever characterised *Genetics and Man* as a "major work on the biology of man" (p95)?

There are, moreover, simple mistakes. DNA is said to have been discovered in 1953 (p20); this is the year in which its structural form was first identified but its chemical composition had been known for almost a century at that time. The inheritance of aryl hydrocarbon hydroxylase as here described (p129), has not been confirmed although the relationship of the inducibility of this enzyme to lung cancer seems still to hold. Although the book is generally well produced from the printer's vantage point, there are a few ludicrous misprints. I'll cite one. The dust jacket identifies William Shockley as Schockley, which in view of the racism of which he is charged, or at best the naive succour of racists ascribed to him, borders on shtick.

I cannot urge the reader of this review to dash out and purchase *Who Do You Think You Are?* for unless he or she belongs to the uncertain audience to which it is addressed he (or she) will be disappointed. As I attempted to imply at the outset, the book could have been more, and Oliver Gillie could have written one which would serve our time importantly. I fear, however, he has pandered where he should have been principled; has been simplistic where he should have been simple; has polarised where he should have been understanding; and has generally failed to appreciate the power of satire, if his intent is to serve a particular cause or point of view. I would urge, as a model, he read (or reread) Haldane's *Politics and Heredity*, an opus of the 30s, before he ventures into this arena again. ☐

William J Schull is Director of the Center for Demographic and Population Genetics at the University of Texas Health Science Center, Houston, Texas.

Nucleic acid treatise

Photochemistry and Photobiology of Nucleic Acids Edited by Shih Yi Wang Vol. 1: Chemistry. Pp xv+576. (Academic: New York and London, 1976.) $54, £29.50.

THIS is the first of a two-volume treatise the purpose of which is to summarise our present knowledge of the photochemistry and photobiology of nucleic acids. The present first volume deals with the physical and chemical processes induced by absorption of a photon in nucleic bases—including rare and synthetic ones—as well as their monomeric and polymeric derivatives. The second volume is devoted to the DNA photochemistry and the photobiological aspects.

Each chapter is written by a group of workers, who are specialists in their respective fields The basic concepts in the domain of photochemistry are first outlined, followed by a complete and up-to-date review on the excited states of nucleic acids. Emphasis is, of course, placed on the new and exciting developments concerning room temperature emission.

The main core of the volume is the description of those photoproducts known so far Particularly pertinent is the analysis of the photohydrates and of the cyclobutane-type photodimers. The volume succeeds in presenting a balanced point of view on conflicting datas or interpretations coming from different groups, and discloses to the reader the problems that remain to be resolved.

Interesting chapters are devoted to the other pyrimidine adducts, the list of which is rapidly expanding, and to the purine photochemistry. In particular, the spectroscopic and photochemical properties of nucleic acids in frozen aqueous solution are reviewed. Finally, there are three chapters concerned with the methodology which has proved to be so useful in establishing the chemical structure and detailed conformation of the photoproducts—mass and nuclear magnetic resonance spectrometry, and X-ray crystallography. Also included is a chapter on radiation chemistry.

The publication of this book is particularly opportune, coming at a time when the repair mechanisms of nucleic acids are so extensively studied. It should therefore serve as an important source of references. Some aspects are redundantly treated, and some others of peripheral interest to the authors are briefly and in a few cases inaccurately mentioned. More fundamentally I would have expected a more integrated presentation of the chemical and biological data for a book intended to be a classical treatise.

Alain Favre

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View from space

Earth Sciences in the Age of the Satellite. By J. Pouquet. Pp. ix+169. (D. Reidel Dordrecht and Boston, Massachusetts, 1976.) Dfl.58; $22.50.

THE view from space of the Earth's surface is providing a distinctive new source of environmental information. This distinctiveness is based firstly on the overview of large areas which makes possible the detection of large scale features that cannot be easily recognised either on the ground or from aircraft, and secondly on the possibility of monitoring rapidly changing phenomena using the regular repetitive imagery obtained from many satellites. Although imagery from satellites has been available for some years, many earth scientists have been rightly sceptical of (perhaps because of) the over-optimistic claims made for remote sensing. The more cautious, more carefully considered study of remote sensing that is now becoming increasingly common is leading many earth scientists to examine its potential, not as a panacea, but as one of several alternative data sources.

Earth Sciences in the Age of the Satellite, was first published in 1971, and has more recently been published in an updated form in this translation. This may well account for many of the naive hopes expressed about the role of remote sensing and satellites.

In the first of the book's three parts, properties of radiation from various parts of the electromagnetic spectrum and their interaction with the Earth and atmosphere are briefly described. The second part on the role of satellites is out of date, although it is a very useful compilation of information about the earlier satellites. The final part concentrates on the applications to the earth sciences, with greatest emphasis being placed on geopedological examples (*sic*). Many of the examples are superficial or even trivial and will convince few that remote sensing has any substantial potential. No proper evaluation of the remote sensing methods is presented, nor is there any sensible objective comparison of their merits relative to other more conventional data gathering methods. In this and previous sections, the illustrations are often unhelpful and more and better photographic reproductions are clearly needed.

The idiosyncratic style of writing will be annoying to most readers and does not merely arise as a result of poor translation. Finally, the book is costly (£14.50) considering its length (169 pages), and fortunately there are other much better books now available.

J. R. G. Townshend

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Infrared and Raman spectroscopy

Infrared and Raman Spectroscopy. Part A. Edited by E. G. Brame and J. Grasselli. Pp. ix+345. (Dekker: New York and Basel, 1976.)

THIS is the first of a projected set of three volumes on infrared and Raman spectroscopy. The set is intended to cover all possible applications of these techniques in such diverse fields as the food industry, petroleum, textiles, biology, environmental science, and so on. This, the first volume, is more concerned with academic, rather than with industrial uses, but a sound understanding of the basic principles is obviously necessary before these techniques can be properly applied.

The first chapter is an introduction to molecular vibrations by Crawford and Swanson. It introduces the basic concepts in a manner suitable for those new to this field, with all the clarity and good humour one would expect from such a source. The chapter covers the origins of infrared and Raman spectra, and the experimental techniques, and introduces such concepts as selection rules, coupled oscillators, group frequencies, intensities and quantitative analysis.

The second chapter by Carter deals with inorganic materials. This delves more deeply into group theory, symmetry, and the selection rules, and shows in detail how the infrared and Raman spectra can be used to derive the structures of some specific inorganic molecules. It deals also with site-and-factor group analysis and with the spectra of oriented crystals. It concludes with some useful comments on experimental methods and with some character and correlation tables.

Chapter three, by Edgell, covers the vibrational analysis of organometallics. It extends the discussion of the selection rules to cover the concept of hidden symmetry, and again illustrates the application of the technique to a number of specific molecules. Finally, it discusses the derivation and significance of force constants in molecules of this kind.

Chapter Four, also by Edgell, is on ionic organometallic compounds. This deals almost entirely with a relatively new field, as the bands arising from the translational modes of ions in solution were not observed until 1965. The author shows that the study of the translational and intramolecular vibration bands can yield some very valuable information on the structure of solutions, on the motions in them and on the nature of the short range forces involved. This is a very highly specialised topic and includes some previously unpublished research by the author Nevertheless it is a topic which is very properly included if the whole work is to meet its stated objective of covering all possible applications.

It is all too common to find that texts written by a number of different authors contain a good deal of overlap and repetition. It is therefore a pleasure to be able to report that this is not so in this case. The editors have done an excellent job of integration so that topics such as the selection rules are introduced in a preliminary way in the first chapter, carried further in the second and yet further in the third. The text is well presented, the diagrams are clear and the standards of proof reading have been high. I could detect only one error, the misplacing of DCN in Fig. 4.

New entrants to this field will find the first chapter comfortingly simple and non-mathematical, but they must be prepared for increasing complexity as the book proceeds. This is however, a necessary requirement for the academic topics covered and they should not feel deterred from exploring the future volumes if their interests are primarily in industrial applications. Altogether this is an excellent start to what promises to be a very good and authorative series.

L. J. Bellamy

L. J Bellamy, formerly Director of the Explosives Research and Development Establishment, Ministry of Defence, is Visiting Professor at the University of East Anglia, Norwich, UK.

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Thermodynamics and surface chemistry

Equilibrium in Solutions; Surface and Colloid Chemistry. By G. Scatchard. Pp. xxxv+306. (Harvard University: Cambridge, Massachusetts and London, 1976.) £20.40.

THE foundations of theoretical thermodynamics laid by Willard Gibbs represent a truly great intellectual and artistic achievement, perhaps marred a little for most of us by the feeling that it seems to require almost as much effort to understand or use or teach thermodynamics as we imagine to have been required for the original creation. George Scatchard devoted a lifetime of effort to the subject, understood it better than anyone else, and used it for a series of definitive research papers on the thermodynamics of complex systems, often systems of biochemical interest. He also taught a graduate course on the thermodynamics of solutions at MIT and an undergraduate course on surface and colloid chemistry. This book contains the text of lecture notes for these courses, originally prepared around 1950 and made available to students in mimeographed form. They were corrected by Scatchard before his death in 1973 and published in the present form with the aid of a grant from the Commonwealth Fund. Walter Stockmayer is quoted in the preface as stating that publication of a text 20 years after it is written "implies that it is unique and in some measure timeless", an assessment with which this reviewer fully concurs.

The goal of the section on *Equilibrium in Solutions* reflects the attitudes of the author's research papers. It is to be meticulously correct in theoretical concepts and equations, and at the same time to be completely practical in the sense that the discussion always focuses on real experimental data and procedures There is no patience with theoretical "ideas" that do not relate to experimental measurement; on the other hand, when measurements are *more precise* than theory, then the theory must be empirically extended and it is important that the extension does not violate physical laws in simple limiting situations.

All the chapters are in a highly compressed style, difficult to read unless one is already somewhat familiar with the subject But it is very rewarding once one gets the hang of it because all the essentials are actually there; in fact they stand out with greater clarity than they generally do in more comprehensive texts.

Electrolyte solutions are treated in greater depth than other topics, and the treatment covers ion transport as well as equilibrium properties. The problems inherent to a definition of single ion activities are discussed, as are the various ways of extending the Debye–Huckel limiting law to higher concentrations. On the other hand, there is no mention of polyelectrolytes. Chemical equilibria are treated with extreme brevity (little more than a definition) and the reader will find no mention of practical aspects of treating chemical equilibria, such as are involved in 'Scatchard plots'.

The *Surface and Colloid Chemistry* section has six chapters: Introduction; Surface tension and interfacial tension; Polycomponent field surfaces; Adsorption on solids; Physical properties of colloid solutions, Electric effects at surfaces and in colloid solutions. It is even more compressed than *Equilibrium in Solutions*, being only 67 pages long, but again the essentials are there, with the notable exception that micellisation and other self-association processes are not considered. There are no literature references, so that these notes need to be read together with more modern texts (the plural is used advisably here because at least two separate texts will be needed to cover the material).

In addition to the scientific texts, we are provided with an appreciative introduction by I. Herbert Scheinberg, an autobiographical note, and a list of Scatchard's publications. The major portion of the autobiography consists of a brief explanation for every paper listed in the bibliography, as befits a man for whom work was clearly a labour of love. Because of his own deep involvement, he had no patience with superficial science, and he labels a number of his papers as "polemics", designed to expose shallow thinking and fallacy. He also used book reviews as a vehicle for criticism, and both the reviews and the polemics are useful reading because the fundamental core of a subject, that Scatchard was always striving to reach, sometimes emerges especially clearly in relation to a point of contention.

This volume will have strong appeal to all who love to shape equations into a description of the real world (in preference to pictures), for it is the record of a man with unique facility for doing so. It also should be read by anyone who ever has to teach thermodynamics or surface chemistry, for it cannot help but sharpen his insight and ability to transmit it.

Charles Tanford

Charles Tanford is James B. Duke Professor of Physical Biochemistry at the Duke University Medical Center, Durham, North Carolina.

H. Mohr
Lectures on Structure and Significance of Science
Approx. 22 figures. Approx. 280 pages. 1977
Approx. DM 29,80; approx. US $ 13.20
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Hans Mohr is a natural scientist with a deep-rooted interest in the nature of scientific thought and in the significance of science. His first book on this subject entitled „Wissenschaft und menschliche Existenz" was written in German (2nd edition, 1970). The present text is based on a series of 15 lectures which the author delivered at the University of Massachusetts during the fall term of 1975. In the Prologue "Science and Responsibility", some basic problems are formulated in more detail. While the treatment of the "Scientific Method" can already claim a well-established tradition of scholarship, the responsibility of the scientist has up to now only rarely been used in the consideration of the phenomenon of science. The author does not equate philosophy of science with epistemology, nor does he follow the tendency of excluding anything from consideration which might raise moral problems. Rather, the lectures on "The Ethics of Science", "The Crisis of Science" and "Science and Values" are as essential for the book as the epistemological treatment of "The Scientific Approach". Another characteristic of the book is that some traditionally epistemological problems, such as empirism and rationalism, are investigated from the point of view of scientific knowledge.

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Mammalian peripheral nervous system

The Peripheral Nerve. Edited by D. N. Landon. Pp. xii+836. (Chapman and Hall: London, 1976.) £25.

VARIOUS aspects of present knowledge about the mammalian peripheral nervous system are reviewed in this book, special emphasis being placed on fine structure, and on the nature of the relationships that exist between the neurone and its supporting cells, end-organs, and other surrounding tissues.

The first nine chapters deal with the functional morphology of the myelinated nerve fibre (D. N. Landon and S. Hall); unmyelinated nerve fibre (J. Ochoa); perineurium and connective tissue (F. N. Low); sensory ganglia (A. R. Lieberman); anterior horn motoneuron (S. Conradi); spinal and cranial nerve roots (H. J. Gamble); ganglia of the autonomic nervous system (G. Gabella); sensory terminals (L. H. Bannister); and motor end-plates (G. F. Gauthier). The remaining chapters review motor end-plate function (A. J. Buller); chemistry and structure of myelin (N. A. Gregson); histochemistry of peripheral nerves and nerve terminals (J. F. Hallpike); selected aspects of peripheral nerve pathology (G. Allt); and electrophysiological properties of peripheral nerve (J. J. B. Jack).

This comprehensive survey, with its extensive bibliographies and superb illustrations, will provide a valuable reference work for those engaged in research on the mammalian peripheral nervous system. Naturally, in a work of such length and scope and varied style some parts appeal more than others For me the authoritative reviews on myelinated and unmyelinated axons, and on the nerve sheaths, are among the most interesting and useful contributions. There is also an excellent account of Wallerian degeneration and subsequent regeneration (excluding re-innervation), and an admirably comprehensive review of receptors.

The editor is to be congratulated for embarking on such a worthwhile enterprise and bringing it to a successful conclusion. There are signs, however, that active and critical editorship was sometimes lacking. For example, the consecutive chapters on motor end-plate structure and function should have been edited so that one complements the other. As it is, no functional comment is made on much of the ultrastructural information; "tonic" and "slow" muscles in one are respectively "slow" and "slow-twitch" in the other; references to "Normanski" optics in the chapter on function remain uncorrected; and the use of the term *"en grappe"* is left in unnecessary confusion. Another contributor is allowed to depict a periaxial space in the Golgi tendon organ, and to present a bizarre account of muscle-spindle innervation in which, for example, the secondary sensory endings are supplied by "group IIa" afferents, and a collateral motor innervation is derived from axons belonging to "group IIb". The editor certainly knows better than this; perhaps it is his view that an editor should do little more than serve as the channel through which his sources flow freely and unfiltered from pen to print. At times that way can leave the reader floundering.

The book, over 800 pages long, is heavy (1.5 kg) to handle and at times heavy to digest. Paragraphs sometimes run on far too long and occasionally there is a rash of instructional italics. Publication evidently took three years, writing being mostly completed by 1973, although an effort has been made to update references to 1975. It might have been better to have published the work in two volumes; better still to have included other aspects and run to three. The value of a well-documented survey of this standard would certainly have warranted that.

David Barker

David Barker is Professor of Zoology at the University of Durham, UK.

Heat on the move

Thermal Conduction in Solids. (Oxford Studies in Physics.) By R. Berman. Pp. xi+193. (Clarendon: Oxford; Oxford University: London, December 1976.) £9.75.

EVERY now and again one happens gratefully on a monograph born purely of the author's desire to communicate, to distil the fruits of a lifetime's personal interest and research into a form which renders the topic accessible to a wider audience. This is just such a book. Our present relatively mature understanding of the mechanisms of thermal conduction in solids owes much to a steady succession of illuminating experiments carried out during the past thirty years by Dr Berman and his coworkers; so that, being a gifted expositor, he is particularly well fitted to convey to others some of his continuing fascination with this technologically important subject.

The book is aimed mainly at physics graduates (or, indeed, final-year undergraduates) who want to progress beyond the rather limited discussion of thermal conduction usually provided by general textbooks on solid-state physics, but who lack the stamina necessary to tackle the unusually abstruse symbolism of the monumental work by J. M. Ziman (*Electrons and Phonons*, Clarendon, 1960). Throughout, Dr Berman's emphasis is very much towards conveying an understanding of the topic in question, and he has eschewed the algebraic pendantries which, in the name of rigour, can so easily obscure the physics for most readers. The dustcover's description of his treatment as "detailed but uncomplicated" is entirely apt.

The book opens with an explanation of what the thermal conductivity coefficient is, how it is measured, and the general way in which it behaves for a variety of different materials. The next six chapters, dealing with conduction in non-metals, include discussions of phonons, phonon-phonon interactions, the scattering of phonons by defects, the Boltzmann equation and methods for solving it; and they explain how these concepts and techniques are able to account for the features of conduction observed in nearly perfect crystals, imperfect crystals, and amorphous materials. Of particular note are the sections which explain the subtle influence of normal (momentum-conserving) processes, Poiseuille flow of the phonon gas and a number of interesting resonant phonon-scattering mechanisms. These are topics which in most textbooks are dealt with either cursorily or not at all. The second half of the book deals lucidly with situations in which heat is transported by electrons, discussing the effect on the conductivity of various types of electron scattering in metals and alloys including superconductors, and in semiconducting materials.

The deliberate emphasis towards a description of work below room temperature is justified by the fact that such experiments usually provide a more sensitive test of the theory than is afforded by those carried out at higher temperatures. An extensive set of references to original papers is provided, and will enable readers to follow up particular aspects of earlier work which are of interest or of relevance to their own research. Books and review articles are also cited where they are likely to be helpful.

Dr Berman's well integrated and readable little monograph has filled what—with hindsight—was an obvious lacuna in the literature. It will continue to be of value for many years to come.

P. V. E. McClintock

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obituary

Sir David Martin

SIR DAVID CHRISTIE MARTIN, the Executive Secretary of the Royal Society, died suddenly on Thursday 16 December 1976, after nearly thirty years' service as the principal executive officer of the Society. Born on 7 October 1914 in Kirkcaldy (where he did an early morning paper round which inured him to a lifelong regimen of early rising), and starting from a local primary school he proceeded to Kirkcaldy High School and thence in 1933 to Edinburgh University. After first class honours in chemistry in 1937, he took a Ph.D. in 1939 under the supervision of J. A. V. Butler on organic compounds containing deuterium. His flair for administration had already shown itself: he was the first President of the Science Students' Union at King's Buildings, and he was much concerned in the Students' Representative Council and the Athletic Club. Originally intending to become a school teacher, but having had his horizons broadened by research and by student activities, he became in September 1939 Assistant Secretary of the Royal Society of Arts, which immediately released him for service in the Research Department of the Ministry of Supply for the duration of the war.

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This last experience convinced him of the contribution that science could make to human affairs, even though he noted that altruism and an ability in science were not always linked characteristics in human make-up. His success in administration brought him to the notice of senior scientists, and resulted in his appointment in 1945 as General Secretary of the Chemical Society and in 1947—at the age of 32—as Assistant Secretary of the Royal Society. In 1943 he had married Jean MacGaradh Wilson; and throughout his life their home, first at Stanmore and then in the new premises of the Royal Society, was a centre of warm hospitality.

As chief executive of the Society, he found himself administering an annual Parliamentary Grant-in-aid of £36,000 and a staff of 29. Some measure of the growth of his responsibilities (whilst he was not in the least an "empire builder") may be gained from the fact that the corresponding figures at his death were about £2,000,000 and 100. Following the Scientific Information Conference in 1948, which he organised between scientists of the Commonwealth and the U.S.A., he persuaded the Royal Society in the 1950s to become its own publisher, and he encouraged other learned societies to do likewise, a step which proved substantially to their benefit. In the years before 1957 he contributed greatly to the organisation of the International Geophysical Year, in which not only were Soviet scientists brought into co-operation, but the Antarctic Treaty was signed, recognising Antarctica as a scientific laboratory above military or commercial interests.

The years after 1957 were charged with the publication of the results of the International Geophysical Year, and with preparations for the celebration of the Society's Tercentenary in 1960. This started with a formal opening by Her Majesty The Queen before an audience of some four thousand in the Albert Hall on 19 July, which was followed by ten days of lectures and visits, including a Service with a congregation of fifteen hundred in St. Paul's Cathedral. The celebrations were a major national event, with many overseas guests who had to be looked after in a manner matching the occasion, and their success was largely due to the efforts of Martin and his relatively small staff.

For some years it had been evident that the activities of the Society were out-growing its premises in Burlington House, and the possibilities of a move were contemplated. There had been talk of a national science centre somewhere in London where all scientific societies could be brought together, but the proposal that eventually took shape was the move of the Royal Society into new premises in Carlton House Terrace. This was very largely achieved under the Presidency of Lord Florey by Martin, the move finally taking place in 1967 under the Presidency of Professor (later Lord) Blackett. In this way the Society was enabled to stay in most fitting premises in central London; and although these could not become the nucleus of a national science centre, the generous policy of the Society in making its accommodation available to other learned societies for symposia and conferences has helped to make it even more a forum of general scientific activity than it was previously.

The large post-war growth in the Society's activities coincided with Martin's appointment. It was primarily due to the greater national appreciation of the value of science and technology resulting from their effects in World War II. As the time of his appointment the number of Fellows elected annually had just been raised from 20 to 25, and it was increased to 30 in 1965 mainly to give increased recognition to engineering and technology, and to 40 in 1975, partly to recognise the growth of science and technology in the Commonwealth. Martin made a characteristic contribution in effecting all aspects of the expansion so smoothly that very few growing pains were encountered, and the staff that he recruited to support him have unfailingly reflected the courtesy, helpfulness and discretion of which he was a model.

In addition to the obvious landmarks in the evolution of the Royal Society in the past thirty years there have been many other new activities for which he provided enthusiastic support. Relations have been developed and maintained with a wide range of national academies overseas, including those in China, the Commonwealth, Japan, Latin America, the Middle East, the U.S.A., and the U.S.S.R. The International Council of Scientific Unions, too, owes much to Martin's support, as does the European Scientific Exchange Scheme, in which some 90 British scientists are exchanged annually with a corresponding number from the Continent; and in the 1960s he did much to save Aldabra and promote the foundation of the Royal Society research station there. These great peaks of administration, however, convey little idea of the continuous demands made of him in running the many activities of the Society. It has some 120 committees and he was Secretary of nearly all of them. He also attended many of the scientific lectures, for he always retained his interest in science for its own sake.

Ever since Babbage's celebrated attack on the Society in 1830 in his *Reflections on the Decline of Science in England*, the function of the Society has from time to time been questioned. I can remember Martin's chuckle when I drew his attention to the fact that his 1830 predecessor had come directly under Babbage's attack for taking a 10% commission from the keeper of the tavern in which he persuaded the Royal Society to hold its annual dinner. Martin's own quiet good humour contributed to that sense of proportion which he always maintained, both in dealing with the occasional challenges to the Society and with the idiosyncracies of some of its Fellows.

One result of Babbage's attack, incidentally, was that the British Association was formed in order to become the main forum for professional men of science, in contrast to the Society, to which laymen were often elected at that time. Over the years, the functions of the two bodies have tended to exchange, and one of Martin's further interests lay in the Association; he was a member of its Council almost continuously from 1959 onwards. Once again, he played a part in getting Government support for the British Association, with funds that have been channelled through the Society.

His abilities were very widely drawn upon. He was a member of the Councils of the Royal Society of Arts, the Society for the Protection of Science and Learning, and the Charities Aid Foundation; and he was on the Executive Council of the CIBA Foundation. The Royal Society of Edinburgh elected him a Fellow in 1956. Since 1967 he had been Chairman of the B.B.C. Science Consultative Group, and since 1950 a Commissioner of Income Tax. His work was recognised by the award of a C.B.E. in 1960, and a Knighthood in 1970, and Honorary Degrees of D.Sc. from the University of Edinburgh and of D.C.L. from Newcastle.

But with all his public service, the Royal Society—both for itself and what he believed it could do—was his absorbing interest. It recognised the increased responsibilities of his office when it re-designated him Executive Secretary in 1962. By a Statute of 1847 he could not be a Fellow, for in a sense his post is one in which the holder has to be a man apart from any of the sectional interests that constitute the full spectrum of science: "A means for the Fellows to express their policies" as he himself put it, adding "I have tried to organise affairs so that all the decisions on scientific matters are taken by the Fellows." To him, his part lay in securing all the information that they needed before taking a decision, and in smoothly implementing the decision once it was taken. He was careful not to volunteer guidance but members of Council, and many of its committee chairmen, gratefully found that he always had something helpful to say when he was asked for his opinion.

Although he could not be a Fellow, few Fellows have done more or had greater influence for good in the Society since World War II than David Martin. With his fervent belief in its cause, his valedictory words are a message for the future: "As the years have passed I have become more and more convinced that it is not enough to leave the advancement of science to scientists alone. The need to see it as making a contribution, and only one contribution, to the world's problems has caused me to recognise the desirability of seeking liaison with other bodies. . . . These bodies operate in different or wider canvasses and, in my belief, operate better in liaison with the scientific activities which the Royal Society represents. The ivory tower concept for scientists is now anachronistic and although I feel an elitist organisation for science has a vital and important role, the base of the pyramid of excellence must reside in the ordinary everyday life of the people."

As for a personal appreciation, what he said of Harold Hartley on the latter's 90th birthday might well have been said of himself: "I would not dissent from a eulogy that ascribed to him the philosophical powers of a Socrates, the strength of a Hercules and the decisive action of a Churchill, but above all he has a warm humanity, which secures a place of affection in the hearts of all those privileged to know him as a friend."

R. V. Jones

Leopold Ruzicka

LEOPOLD RUZICKA, one of the greatest and most influential organic chemists, died on September 26, 1976. His work exemplifies that combination of severely disciplined empiricism wtih intellectual fantasy and imagination, which is the hallmark of first class organic chemistry. Ruzicka moreover was a unique character with a personality which was perpetually interesting and stimulating to all who knew him. His life also could teach us a lesson sorely needed in the United Kingdom—how to make an effective combination of academic and industrial efforts in research—if only those in authority would pay heed.

Ruzicka was born on September 13, 1887, in Vukovar, a small town in Eastern Croatia where the river Drava joins the Danube. The family name is Czech, going back to his great-grandfather. Ruzicka's father, a cooper and timber merchant, died when the boy was four years old, and the family moved to the neighbouring town of Osijek, where the young Leopold attended the classical high school, learning much Latin and Greek but neither chemistry nor geometry. At an early age Ruzicka was determined to become a priest, but this ambition had to be abandoned and, after much thought, he decided that his life's work should be in the organic chemistry of natural products. The circumstances which influenced this decision, remarkable at such an early age, are obscure; but we have Ruzicka's own word for it.

At the age of 18 Ruzicka left the Austro-Hungarian domain and sought training in Switzerland or Germany. His first aim was to enter the Polytechnic in Zürich, but the entrance examination was forbidding in its requirement for geometry as well as chemistry. So Leopold went to the Technische Hochschule at Karlsruhe, where he was accepted without examinations. The choice was fortunate because he was free to select the lectures suited to his special interests, and he joined Hermann Staudinger. It is typical of Ruzicka's extraordinary energy and intellectual ability that in only four years he completed the course for both first and higher degrees, graduating in 1910 as Doctor Ingenieur. Staudinger invited his outstanding pupil to join him in investigating the then unknown constituents, poisonous to insects, of *Chrysanthemum cinerariifolium*, the pyrethrins now of such economic importance. And in 1912 Staudinger succeeded Richard Willstätter at the Eidgenössische Technische Hochschule, Zürich. So Ruzicka arrived eventually, without examination, at the institute which he was to serve so well for many years. In 1916 he ceased collaboration with Staudinger in order to work on his habilitation thesis, which in 1918 brought him the rank (unpaid) of Privat Dozent and in 1923 of titular Professor. His researches were in the field of alicyclic compounds, which always remained his chemical interest.

Finance was a great problem to the unpaid lecturer, and in 1921 he accepted an invitation to collaborate with the perfume factory in Geneva, then Naef et Cie and nowadays Firmenich et Cie. So began an academic-industrial collaboration, fruitful for both partners. Ruzicka's research proposal was 'synthesis of the sesquiterpene perfumes farnesol and nerolidol; clarification of the structure of the four important odorous ketones, civetone, muscone, irone, and jasmone'. Until 1925 the work was carried out in Zürich in a cellar, with eventually eight co-workers, but then it was transferred to better quarters in Geneva. It was one of the classical, seminal investigations in organic chemistry. Nowadays it is difficult to remember that accepted dogma precluded stability for macrocyclic carbon structures, such as were proved to be the basis of the commercially important perfumes of musk and civet. The structures of the natural products were elucidated, they were synthesised, and a simpler, cheaper synthetic musk perfume, 'exaltone', was produced. Ruzicka spent only 18 months in the industrial laboratory, but that cemented a lasting relationship between it and the ETH, Zürich.

In 1926 Ruzicka accepted a call to the Professorship in Utrecht, where he pursued the chemistry of the many membered rings and of the higher ketones. In addition he began to acquire that deep love and knowledge of Dutch painting, which was to become almost as important as his love for chemistry. The stay was, however, short because Staudinger's successor at the ETH, Richard Kuhn, returned to Germany, and the President of the ETH, Rohn, came to Utrecht to offer the post to Ruzicka. Thus Ruzicka, who had became a Swiss citizen in 1917, became the first non-German to occupy the chair, although in due course he continued their tradition, still maintained by his own successor, of becoming a Nobel Laureate.

At the ETH Ruzicka threw himself into the task of making it the leading organic chemical institute in the whole world. He built so well that this position has, in the perhaps prejudiced view of the writer, never been lost. Ruzicka had an oft-quoted saying, "academic freedom consists in being allowed to work far harder than is prescribed." He certainly followed his own precept —and made similar demands on his colleagues. From the Zürich laboratory poured many publications opening up the detailed chemistry of the higher terpenes, the steroids and especially the sex hormones. The series of papers *Ueber Steroide und Sexualhormone*, continued by former collaborators, has reached part 256 and another series *Ueber Triterpene* likewise reached part 196. Ruzicka took pride in the way in which these marathons were continued by relays of his pupils. The triterpene series, notable for the skill and exactitude shown in unravelling immensely difficult problems, was joint work with O. Jeger. Jeger went on to investigate the photochemistry of alicyclic compounds, and yet another long series of papers emerged and yet another first-class chemist, a "chemical grandson" of Ruzicka, K. Schaffner, now at the Max-Planck Institute, Mülheim.

Ruzicka had an instinct for the significance of apparently minor natural products. Few could have discerned that the constituents of wool fat, especially lanosterol, would turn out to have great importance as the precursor of the steroids.

In this work Ruzicka shrewdly enlisted financial support from both the CIBA company in Basle and the Rockefeller Foundation. The close contact with CIBA included supply of personnel for their laboratories and assignment of patents, which proved to be profitable. It is worth noting in passing that Ruzicka's successor, Vladimir Prelog, became a member of the board of CIBA, now CIBA–GEIGY.

While much of these researches comprised detailed experimental exploration of complex organic structures, the organic chemist's characteristic underlying interest in correlating experimental data was never neglected. The isoprenoid origin—in modern terms derived from mevalonate—of these natural products was always in Ruzicka's mind and eventually there emerged the biogenetic isoprene rule. Such interests naturally led Ruzicka towards biochemistry proper, and he was largely instrumental in the establishment of a biochemical institute in the ETH. Moreover his younger colleagues, notably V. Prelog and D. Arigoni, have extended the scope of organic chemical researches into enzymology and experimental, as distinct from merely speculative biosynthesis.

In his prime Ruzicka had a rather conservative view of organic chemical theory, but again it is noteworthy that another of his brilliant pupils, A. Eschenmoser, has transformed much of our outlook on organic chemical synthesis by thorough understanding of theoretical concepts.

Initially Ruzicka had a critical view of physical chemistry, but, as his interest in spectroscopic and other physical methods grew—and he ensured that his institute lacked nothing in instrumentation in that regard—his sympathy for physical chemistry increased. He helped the growth of young physical chemists and the development of a separate institute in the ETH.

The patents on partial synthesis of the male hormones, testosterone and methyltestosterone, generated substantial sums of money. Ruzicka used them to built up a superb collection of Dutch painting—works by Franz Hals, Rembrandt, Rubens, and many others—which was presented to the Zürich Kunsthaus. One example of the quality of this collection will suffice: a Dutch art expert considered that of all the views of Haarlem painted by Jacob van Ruisdael, the finest was in Ruzicka's collection. This collection was not assembled by merely following the advice of art dealers. On the contrary, Ruzicka was a very knowledgeable amateur, in the best sense of the word, and his Zürich endowment includes an important library. The former director of the Mauritshuis in the Hague has testified to Ruzicka's expertise and his characteristic cunning and energy in acquisition of the best pictures for his collection. The writer remembers Ruzicka's indignation when difficulties were raised about an export licence for the portrait *Philip the Fourth* (Rubens) bought in London. Various letters, one translated into "too polite Cambridge English", another in Ruzicka's own words, were ineffective. Eventually a successful arrangement was made through the diplomacy of the Swiss Ambassador in London.

No memorial to Leopold Ruzicka would be complete without reference to his wit and penchant for entertaining stories. Although a very loyal Swiss citizen, he loved to parade his left wing views, suitably exaggerated for the benefit of the conservative Swiss, while being proud of his membership of the Papal Academy of Sciences, not forgetting his simultaneous membership of the Soviet Academy. It is recorded that in a Leningrad hotel he announced loudly that "as we are all good Party members, we can speak freely". As his hosts blenched, he went on to compare unfavourably the contributions made by Stalin to the Hermitage collection with those derived from Catherine the Great. This outspokenness was evident in the laboratory. The writer well remembers a distinguished American author of a popular textbook on organic chemistry, being distinctly disconcerted by the interruption of his lecture in Zürich because Ruzicka would not tolerate a pentavalent carbon atom on the blackboard of his laboratory. But there was no malice in Leopold Ruzicka. He loved to see his younger colleagues develop and the superb institute at Zürich stands as a lasting memorial to his life and work.

Ruzicka detested Nazism, and he gave generous succour to victims of Nazi oppression.

He received, of course, numerous honours, among them the Nobel Prize (1939) and Foreign Membership of the Royal Society (1942).

The autobiographical memoir *In the borderland between bioorganic chemistry and biochemistry*[1] makes rewarding reading, as does Ruzicka's own obituary notice of Arthur Stoll[2], which reveals much of Ruzicka himself as well as the subject of the memoir.

The writer thanks Professor O. Jeger and V. Prelog for their help in providing information and advice.

G. W. Kenner

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[1] *A. Rev. Biochem.* **43**, 1–20 (1973).

[2] *Biogr. Mem. Fellows R. Soc.* **18**, 567–593 (1972).

R. A. Morton

Richard Alan Morton, Emeritus Professor of Biochemistry in The University of Liverpool, an outstanding British biochemist of his generation, died peacefully at his home on January 21 after a short illness; he was seventy-seven. Born in Liverpool of Welsh parents, Professor Morton received his early education at Oulton School. After a short time in the Army he entered Liverpool University as an undergraduate in 1919 and, apart from a sabbatical year in the United States as Visiting Professor at Ohio State University in 1931, remained there until he retired in 1966. He graduated in chemistry and worked for his Ph.D. under Professor E. C. C. Baly, a pioneer in the application of spectroscopy to chemical problems; in 1924 he was appointed special lecturer in spectroscopy in the Chemistry Department where he remained until 1944, when, with great foresight, the University elected him Johnston Professor of Biochemistry. He held this chair, the first established chair of biochemistry in the UK, with great distinction for twenty-two years.

Professor Morton's early research work was concerned with relating the absorption spectra of chemical compounds to their structure and earned him the Royal Institute of Chemistry's Meldola Medal in 1930; later he pioneered the application of absorption spectroscopy to biology. This interest was first aroused in 1926 when he collaborated with Professor (later Sir Ian) Heilbron to show that an impurity in cholesterol isolated from cod liver oil and which exhibited absorption bands in the ultra violet region of the spectrum, was the compound which was converted by ultra violet irradiation into the anti-rachitic principle. These were the days before the structure of cholesterol was known, but the active compound eventually turned out to be 7-dehydrocholesterol. He then particularly concerned himself with vitamin A which had two spectroscopic 'labels', an ultra violet absorption band (λ_{max} 328nm) and the blue colour (λ_{max} 617nm) which it gave on treatment in chloroform with a saturated chloroformic solution of antimony trichloride. Critical examination of the colour test led to his discovery of vitamin A_2 which was found particularly in liver oils from fresh water fish. Again, thoughtful assessment of the absorption spectrum of 'retinene', the prosthetic group of the visual pigment rhodopsin, began work which led to his discovery in 1941–1942 that retinene was vitamin A aldehyde (retinal) and that the corresponding compound from porphyropsin from fresh water fish retinas was the aldehyde of vitamin A_2. These penetrating discoveries, which gave studies on the biochemistry of visual pigments a new impetus, resulted in Morton's election to the Fellowship of the Royal Society in 1950.

Other important investigations on vitamin A followed but the later stages of Professor Morton's research career saw two extremely important additional discoveries: the first was ubiquinone, discovered independently at the same time in the United States, and the second was the family of polyprenols. The impact of these discoveries on modern biochemistry was profound. The experts on electron transport could from then on carry out their experiments secure in the knowledge that in the case of ubiquinone they were dealing with a well characterised compound. Later work revealed the importance of polyprenols as carrier compounds particularly in cell wall biosynthesis. It gave Professor Morton great satisfaction in his retirement to follow the detailed elucidation of the biochemical significance of these compounds particularly when these developments were carried out by his old students.

During the second world war Morton was concerned with an investigation organised by The Accessory Food Factors Committee of the MRC to determine the vitamin A requirements of humans. The experiments were carried out on conscientious objectors who volunteered as experimental subjects. This stimulated a life-long interest in nutrition and although he never experimented further in nutrition he read deeply in the subject and made many thoughtful contributions to a number of nutritional working parties. In 1969 he was elected a member of The American Institute of Nutrition.

Under Professor Morton's sympathetic guidance the Department of Biochemistry at Liverpool prospered and his crowning achievement was to persuade the University to build the large new building in which the department is now housed. He developed an effective research school and a remarkably large number of his students now hold positions of high responsibility in places as far apart as India and Canada. Professor Morton's great gifts as a scientist and as a wise counsellor became well known outside the University early in his career and he was constantly in demand for professional committees; he served twice on the Council of the Royal Society, was Chairman of the Biochemical Society and was a member of many government committees. From 1963 to 1968, he held the important position of Chairman of the Committee on Food Additives. He enjoyed writing and editing and was a member of the Editorial Board of the Biochemical Journal for seven years and Chairman of the Publications Board of the Royal Society 1961–1962.

He received honorary degrees from the University of Wales, Trinity College Dublin and The University of Coimbra, where his first research student became Rector. When he retired he was elected an Honorary Member of the Biochemical Society.

In retirement he continued to be as active as ever and he retained his fresh and enthusiastic approach to biochemistry right to the end. Indeed he had only just returned from an international symposium in India and at the time of his death was busy editing the proceedings. He spent a great deal of time on the work of the Oceanography and Fisheries Committee of the Natural Environment Research Council and in 1969 was Royal Society Visiting Professor at The Royal University of Malta. In spite of all this extramural activity he found time to complete two projects close to his heart: one was the *History of the Biochemical Society* (1911–1969) and the other a mammoth two volume new edition of his book *Absorption spectra of Vitamins and Hormones* last published in 1942.

In spite of his great achievements and international renown Professor Morton remained unassuming, friendly and approachable. His life shows that shrewdness and devotion to the demanding career of a scientist need not be divorced from kindness, gentleness and sympathy. His many friends and colleagues will remember Alan Morton as a distinguished scientist, wise in his judgement, humane in his outlook, whose long career brought lustre to biochemistry and to the University of Liverpool.

T. W. Goodwin

A hundred years ago

A bright violet meteor was observed at St Etienne on 11 March at two o'clock in the morning, in the southern part of the horizon. It was travelling with great velocity from west to east. No detonation was heard.

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Where is Federico Alvarez Rojas?

LAST year (*Nature* **263**, 452) we reported on the plight of Maximo Pedro Victoria, an Argentinian nuclear engineer imprisoned and brutally treated for no more obvious reason than that he, as a civilian engineer, had been in dispute several years previously with military scientists over the fuel for a new reactor. It seemed that now the military was all-powerful in Argentina, some old scores were being settled; Dr Victoria, who had been a member of the Board of Directors of the National Institute of Technology, had to be taught a lesson which included being beaten up and losing some of his teeth.

Late in October, Dr Victoria was freed; 'your husband must be a real angel to be let out of gaol', a military officer told his wife. He now lives in Belgium. Maybe, we naively thought, this is a turning point and the Argentinian authorities are beginning to let up on the extremely repressive way in which they have been dealing with subversives, real or imagined. Not so; a report just issued by Amnesty International of an international mission to Argentina (Amnesty Dept A, 55 Theobald's Road, London WC1; £1.00) makes it abundantly clear that despite President Videla's assertion that 'there are no Argentines detained for their political opinions', there could be several thousand political prisoners, most not tried or even charged, and some subjected to unspeakable brutalities.

A particularly alarming aspect of the present troubles is the number of people who disappear or are abducted. Estimates of numbers vary widely but since the military coup in March 1976, at least 2,000 have disappeared; in the last week of May 1976 relatives filed a total of 200 writs of *habeas corpus* in Buenos Aires alone. What happens to these people? Representatives of the Argentinian government pointed out to the mission that they might have chosen to go underground or to emigrate, or they may have been killed in a clash with security forces. This looks like only a part of the story; there seems hardly any doubt that many of those missing were taken into custody and that many of the remainder were killed by right-wing terrorist groups which seem to operate without much hindrance from the government. Indeed, recently the Minister of Foreign Affairs, in comparing left-wing guerrillas with microbes on the social body, described reprisals groups as antibodies which would disappear when the microbes were eliminated.

Among the several hundred whom Amnesty list as having disappeared are several scientists. These include Federico Alvarez Rojas, a physicist at the National Atomic Energy Commission (CNEA), and his wife Hilda Leikis, a computer programmer; Roberto Ardito, an electrical engineer at CNEA, and his wife and children; Ricardo Chidichimo, an Air Force metallurgist; and Antonio Misetich, a CNEA physicist with an international reputation. The case of Federico Alvarez Rojas will illustrate the problem.

Senor Alvarez Rojas, 33 years of age, has worked at CNEA since 1968, most recently as director of the X-ray diffraction laboratory; his wife worked as a programmer at Bairesco S.A. (Burroughs). His father, also Federico Alvarez Rojas, was in Britain for some time as a British Council Scholar and worked in the Soil Mechanics Division of the Building Research Station, Garston. Senor Alvarez Rojas Jr and his wife were abducted by an armed group in the early hours of 1 October 1976; their three children were left behind. Since then vigorous efforts to find them have been made by his father who has issued *habeas corpus* writs and appealed to the authorities, the church and the Red Cross without success. Efforts made through American and British diplomatic channels have been unsuccessful.

What can we do? We have a responsibility as scientists to be concerned about illegal actions against any member of the scientific community whether the person in question is known to us or not. As the recent Council for Science and Society report on scholarly freedom and human rights put it, science is particularly vulnerable to outside interference, and it is clear that there is outside interference of an exceedingly ugly kind in the national laboratories of Argentina. The rapid deterioration in conditions caused by the recent events is soon going to leave the country, in the words of one despairing correspondent, 'inhabited only by labourers and murderers'. Let us hope that this message gets through to the president, General Jorge Videla, to whom it is still worth writing at Balcarce 50, 1064 Buenos Aires, to enquire about the fate of fellow members of the scientific community. □

A fishy business

C. E. Purdom and **A. Preston** assess the state of fish farming and its future promise

FISH farming is arousing widespread interest on the basis of a variety of concepts, some of which are not valid. There is such confusion about the motives of fish farming research that it is necessary to define clearly the role that this form of animal husbandry has within the context of UK food supplies.

Throughout the world, fish farming takes many forms but there are three basic categories:

- sea ranching: fish are reared for release into the sea as juveniles, where they subsequently grow to marketable size;
- extensive production using natural productivity;
- intensive farming which relies on supplied feeds.

Sea ranching has strict limitations, being feasible only where the natural tendency of the fish is to return to the point of release when they are fully grown. Salmon and sturgeon in the United States and the Soviet Union respectively are farmed in this way. There seems little immediate prospect that private enterprise will be stimulated by the vision of a valuable investment swimming off to distant waters.

Extensive cultivation is carried out basically by warm water pond farming. It is by far the most widespread of fish farming systems on a global basis and is found in most areas where space, climate and a local market justify it. Carp (*Cyprinus carpio*) is the archetypal species in pond farming, but a quite spectacular growth occurred in catfish (*Ictalurus punctata*) farming in the southern states of the United States during the 1960s and there has been recent progress with several other species of fish in fresh and brackish waters in south-east Asia.

Pond fish farming depends upon natural productivity and, like any other form of extensive animal husbandry, benefits from pest control, the use of fertilisers and the supply of supplementary feeds. Its productivity is governed by management practices and, of course, by the natural features of the farm. Natural productivity alone is low, perhaps a few hundred kilograms per acre per year at best. Fertilisation and other water management practices can double this but big dividends come only from the use of supplementary feeds. Here is a snag: fish are not efficient users of food materials other than animal protein and fat. Occasional reference is made to the desirability, in a protein saving context, of rearing herbivorous fish. The fact that such species require over 100 kg of feed to make 1 kg of flesh, however, puts them way below the performance of farm ruminants.

Intensive farming is almost wholly artificial. Fish are held in small containers which are commonly circular tanks, linear raceways or net enclosures in tidal waters. Very high stock densities are maintained with a large flow-through of water. At fish densities up to 100 kg m^{-3} all the nutritional requirements must be met in prepared feeds which must contain high levels of animal protein, 40–45% dry weight, moderately high lipid content and vitamin supplements. Such feeds are expensive, but the rationale of intensive farming is to produce high quality protein from low quality protein (quality being defined in terms of acceptability for human consumption). Feed protein is converted into fish protein with an efficiency of about 30% and intensive farming is therefore a net user of protein. The fish most commonly farmed this way are salmon and trout, in fresh or salt water, but large quantities of yellow-tail, a fully marine species, are farmed intensively in Japan.

No cheap protein

It is abundantly clear that fish farming is not a means of producing cheap protein. Whenever fish are farmed, they are high quality, high priced foods. A further view of fish farming is that it is an alternative, or a meaningful supplement, to the yield from sea fisheries. This idea is also invalid on the grounds that the two spheres of activity are of widely differing scales. UK fish landing approach one million tonnes per year: currently, fish farming contributes 2,500 tonnes per year. Assuming a ten-fold increase during the next five years, which is both worthwhile and probable, the scale of fish farming will still be totally inadequate to accommodate the fluctuations in the yield from sea fisheries, which can follow from political or management decisions or from natural causes: these may be measured in hundreds of thousands of tonnes. However, for individual species of fish, for which landings are low, for example turbot (700 tonnes UK landings in 1975) or sole (1,300 tonnes UK landings in 1975), there is some prospect of significantly adding to supplies by farming.

Turbot: no pigment problem

Fish farming in the UK probably will be based on intensive systems for the supply of specific types of fish suitable for these systems and for which a current demand exists. The two species likely to fulfil these requirements are rainbow trout (*Salmo gairdnerii*), which constitutes the bulk of present farm production, and turbot (*Scophthalmus maximus*), which has been researched intensively over the past five years and is currently under pilot scale commercial production.

Research on plaice, sponsored by the Ministry of Agriculture, Fisheries and Food (MAFF) began in the 1950s. This species is no longer thought suitable for cultivation because it is relatively abundant around the UK, has a fairly stable population, and is not a good enough converter of food to sustain an economically viable industry. After much background research, therefore, attention was turned to sole and turbot which are prime fish of high value and in short supply. Mass rearing techniques were quickly established for sole along the lines developed for plaice. Little progress was made with on-growing (that is, rearing hatched fish to marketable size and sexual maturity), because the hatchery-reared juvenile fish were difficult to wean onto prepared diets. The sole is a quiet feeder with nocturnal, browsing habits—the introvert of the flatfish families. Further research in MAFF laboratories was restricted to the weaning phase. Turbot was assessed first in terms of its on-growing capabilities. It passed the test, being voracious, a good protein converter (achieving levels up to 50%), and robust and docile towards anything it could not swallow. Growth rate is then dependent on temperature.

Hatchery techniques

Following the satisfactory outcome of these trials, using small fish collected from the beaches, efforts were made to develop a hatchery technique to raise juveniles from eggs. Plaice and sole larvae were satisfactorily grown

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on a diet of *Artemia* nauplii. Turbot, however, were too small initially to feed on nauplii but they could feed on natural plankton, mussel veligers and the rotifer *Brachionus plicatilis*, the latter being the most suitable. After a few days feeding on rotifers, turbot larvae accepted nauplii but the problems did not end there. Metamorphosed fish were reared for the first time in 1972; about 100 were produced from many thousands of eggs. Steady annual improvements in empirical techniques have since been made, and survival rates up to 25% are now feasible.

Solving the early feeding needs of the larvae, at least at an empirical level, did not remove all the obstacles to successful rearing of turbot. A number of minor problems, 'dropsy' in young larvae, a later lack of swimbladder development and atypical pigment formation at metamorphosis, mostly diminished with increasing experience. A major problem, however, was that, unlike plaice and sole, turbot larvae were not satisfied for long on a diet of *Artemia* nauplii. Progressively larger *Artemia*, grown on unicellular algae, were required by the fish as they grew larger themselves. A weaning process was developed whereby the larvae were switched to prepared foods by using specially designed weaning tanks which kept inanimate food particles moving but did not sweep the fish down the overflow. Rearing turbot larvae is now almost routine but it still requires sophisticated laboratory facilities and further research is needed to clarify scientific aspects and to simplify systems for commercial use.

Sole have similarly been weaned from *Artemia* to prepared diets but for rather different reasons. Earlier work with sole relied on a succession of live foods but these are not suitable for commercial use and they not only delay the problem of weaning but also intensify it: the luxury of live foods appeared to be habit forming. A clear demonstration that sole is suitable for domestication still remains to be made.

Coping with seasonal spawners

A problem with turbot, as with most fish, is that spawning is seasonal. This restricts management options in commercial farming and it also limits the pace at which research can proceed. Most seasonal spawners have light or temperature regulated hormonal clocks and in theory can be persuaded to spawn out of season by the correct manipulation of whatever parameter is appropriate. On the basis of successful work with dab, trials were set up to influence the spawning pattern of turbot, which normally spawns in midsummer. Spawning has been brought forward to March in one series and further trials are aimed at delaying spawning until early winter. The indoor work of hatching and larval rearing could then be completed by spring when the juvenile fish could make use of increasing temperatures outdoors.

Work with flatfish has been aimed at creating a new form of fish farming. Recent commercial investment in this area is a measure of its success and has introduced the provision of advice and assistance to industry through research and development programmes. The growth of trout farming led to a similar demand for R&D programmes and, after a recent review, it was planned that all the MAFF's fishing farming research, except work on infectious diseases, should be located at the Fisheries Laboratory, Lowestoft.

The trout research programme is divided into water management, genetics and feeding strategies. Water is clearly a limiting factor in trout farming and in simple systems about 20,000 gallons are used for each pound of fish produced. But the first limiting factor is oxygen. Water-saving systems employing oxygen or air are under development by industry. Removing the limitation of oxygen increases the levels of carbon dioxide, ammonia, nitrite and nitrate. Limited research on the effects of these and other water quality factors is planned but the principal MAFF investigation will be on the use of sea water for farming trout. This offers immediate scope for large scale increases in production.

Rainbow trout are not naturally anadromous but are successfully reared in sea water in some parts of the world. Smolting does not occur, as in salmon and migratory trout, but the ability of rainbow trout to withstand transfer to sea water increases with their size. Preliminary trials suggest that 30–90 g is the range over which fish may be acclimatised but there is evidence that age may be important and that domesticated strains vary in their tolerance of sea water. Clearly it is important to establish the earliest possible transfer to sea water where portion-sized fish of around 250 g are produced, but for larger fish, for which there is a growing market, the need is not so marked.

The genetic programme is based on the fact that several individual strains of rainbow trout already exist. A number of these strains have been imported with the objective of developing disease-free broodstock for subsequent trials both of the characteristics of the strains themselves and of cross-breeds. Electrophoretic analysis of enzyme polymorphisms suggests that some of the strains are inbred.

Strains differ in the time at which they spawn and it may be possible with trout to supply eggs throughout the year without recourse to environmental manipulation—more difficult for trout than for turbot on account of their relatively low fecundity and the need for large numbers of broodstock. Other genetic aims include selection for larger egg size and for late maturity.

Feeding is by far the most cost sensitive area of trout farming but despite a long history of domestication, there is little standardisation of feeding strategies. Current practice in the UK is to use pelleted feeds but in other countries, much reliance is placed on the use of cheap trash fish, particularly in sea water culture. Routine use is made of such materials within Ministry work but detailed nutritional and logistic assessment remains to be made.

Fish farming as a food producing industry has no other commitment but to operate in a cost-effective way. MAFF research has established a basis for turbot farming and continues to explore this and rainbow trout farming in order to supply industry with scientific advice. ☐

Cages of plaice and turbot in the North Channel

Start from the middle

The results of a year-long study of nuclear power by a distinguished panel were published last week. Colin Norman reports from Washington

In a report likely to have a significant impact on nuclear policy in the United States, and perhaps elsewhere, a panel of distinguished scientists and economists has urged the Carter Administration to bar domestic reprocessing of spent reactor fuel, and to curtail drastically the present crash effort to develop a commercial breeder reactor. The economic justifications advanced for those two programmes are dubious at best, the panel said, and to proceed apace with either of them would seriously hamper attempts to curb the proliferation of nuclear weapons.

With the Carter Administration now in the throes of putting together a comprehensive energy policy, to be unveiled on 20 April, the report* couldn't have come at a more opportune moment. In fact, the panel's conclusions are in harmony with some of President Carter's campaign statements, and it is generally expected that the recommendations will be reflected strongly in Carter's energy policy. In any case, the report is said to have gone down well in the White House, and Carter even took time last week to meet with all 21 panel members to discuss their recommendations.

The product of a year-long study sponsored by the Ford Foundation, the report is an attempt to examine and stitch together the economic, political and social arguments which have been raging over nuclear power in the past few years. The effort was initiated, according to the Ford Foundation's President, McGeorge Bundy, because of "a widespread feeling that this debate was suffering from a shortage of disinterested analysis", and the foundation therefore chose as members of the panel prestigious individuals who were not lined up on either side of the debate. Like the report of the Royal Commission on Environmental Pollution in Britain, the panel's conclusions have therefore gained some authority from the fact that the panel started from a middle-of-the-road position.

The panelists were mostly liberal academics many of whom have been active in arms control issues, and they include two top officials in the Carter Administration—the Secretary of Defense, Harold Brown, and Joseph Nye, deputy Under-Secretary of State (Brown was President of Caltech when he served on the panel and Nye was professor of government at Harvard). Other members included John Sawhill, former Administrator of the Federal Energy Agency, Wolfgang Panofsky, director of the Stanford Linear Accelerator Center, Kenneth Arrow, a Nobel prizewinner and professor of economics at Harvard, and Richard Garwin of IBM. The chairman of the panel was Spurgeon M. Keeny, a senior official of the MITRE Corporation, a think-tank which coordinated the effort.

With such an impressive list of authors, it's not surprising that the study has attracted considerable attention. It should be noted, however, that contrary to some interpretations, it is not an anti-nuclear document. Far from it. Though the panel challenges many of the chief arguments put forward by the nuclear industry, and opposes some programmes which the industry believes are crucial for its long-term prospects, the report nevertheless acknowledges that uranium is likely to be an important source of energy for several decades and it suggests that in some respects nuclear power may hold a slight edge over alternative energy sources.

The panel takes issue with conventional nuclear wisdom on two points fundamental to the nuclear debate, however. It argues that, in the United States at least, although nuclear power seems to hold a slight economic advantage over coal, "the choice is so close and the uncertainties sufficiently large that the balance could easily shift to increase or eliminate" that advantage. Similarly, adverse health and social effects associated with nuclear power are likely to be less than those associated with coal, but "the range of uncertainty in social costs is so great that the balance between coal and nuclear power could be tipped in either direction".

That is a far cry from the industry's contention that the atom would supply significantly cheaper and cleaner energy than alternative sources, but it leads the panel to suggest that the United States should maintain a mix between coal and nuclear power. The recommendation is really an acknowledgment of what is likely to happen anyway.

The panel is, however, much less equivocal in its conclusions concerning plutonium. Citing nuclear proliferation as "the most serious danger associated with nuclear power", the panel urges the Carter Administration to renounce domestic reprocessing of spent reactor fuel and recycling of plutonium. "If a decision to postpone this technology indefinitely is articulated and carried out effectively", the panel argues, "it can have a major influence on the assessment of costs and benefits of reprocessing and recycle by other countries that are, or soon will be, facing similar decisions. Conservsely, a US decision to go ahead with reprocessing . . . would accelerate worldwide interest in the plutonium fuel cycle and undercut efforts to limit nuclear weapons proliferation".

In any case, the panel argues that reprocessing is difficult to justify even on economic grounds. The nuclear industry has long argued that reprocessing and plutonium recycle will be needed to stretch out dwindling supplies of uranium and to pave the way for plutonium-producing breeder reactors, but the panel takes issue with the industry's chief assumptions. "Our review convinces us that current official estimates of uranium reserves and resources substantially underestimate the amounts of uranium that will be available at competitive costs", it notes, and suggests that "there will be enough uranium at costs of $40 (1976 dollars) per pound to fuel light water reactors through this century and, at costs of $40 to $70 per round, well into the next century". Moreover, the panel points out that the estimated costs of reprocessing have escalated rapidly, with the result that "any net economic benefit during this century is questionable".

The panel therefore urges Carter to short-circuit a major review by the Nuclear Regulatory Commission of whether reprocessing should be permitted by announcing that the United States will defer use of the technology indefinitely. In fact, Carter promised during his election campaign that he would "seek to withold authority for commercial reprocessing until the need for, the economics and safety of this technology is clearly demonstrated", and the panel is therefore essentially urging him to keep his campaign pledge.

As for the breeder reactor programme, the panel again takes issue with the industry's economic arguments, suggesting that "the early economic potential of the breeder has been significantly overstated". The report also notes that a breeder programme would involve full-scale commitment to the plutonium cycle with its attendant proliferation problems. In spite of those drawbacks, however, the panel doesn't recommend complete abandonment of the programme, but suggests instead that the effort should be sharply reduced in priority and be pursued only as "insurance" against the possibility of very high future energy costs or the failure of efforts to develop alternative energy sources.

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*Nuclear Power Issues and Choices (Ballinger Publishing Co., Cambridge, Mass. $6.95.)

The effort to develop a commercial breeder reactor has long enjoyed pride of place as the federal government's most expensive and most controversial energy research and development programme. The expected cost of the programme has soared from $2,000 million to $12,000 million in the past few years, and the projected date for introduction of commercial breeder reactors has slipped from the late 1980s to the mid 1990s. A small test reactor, the Fast Flux Test Facility, is now nearing completion in Washington State and it is expected to start operations in 1980. The plan is to follow that with a 380 MW demonstration reactor, construction of which was expected to begin on the Clinch River in Tennessee this year, with operation scheduled for 1983. The next step would be a large prototype reactor, funded chiefly by electricity utilities, with construction scheduled to begin in 1981 and operation in 1988. A decision on whether to proceed from the prototype to a full-scale commercial programme would be made in 1986.

The Ford Foundation study suggests that the overlapping, crash programme leading to a decision on commercialisation as early as 1986 is unwarranted, and it recommends that the effort be completely revamped with more emphasis placed on technology development. In particular, the panel recommends that the Clinch River plant be abandoned, and it suggests that the decision on commercialisation can safely be postponed beyond the end of the century.

Again, that recommendation meshes neatly with Carter's campaign pledge to reduce the breeder programme to a "relatively low priority, possibly multinational effort". Last month, moreover, Carter recommended that nearly $200 million be cut from the Ford Administration's budget request for the breeder programme, and he ordered the Energy Research and Development Administration to undertake an intensive study of the need for and timing of the effort. That study is now under way, for ERDA established last month a committee containing some of the breeder programme's most vocal supporters and critics, and asked it to come up with recommendations before Carter's 20 April energy statement.

The Ford Foundation panel also urges the Carter Administration to take a number of other steps to reduce pressures leading to nuclear proliferation. In particular, it suggests that "the United States must have a clear policy on its long-term role in providing enriched fuel to both domestic and foreign nuclear power programs". The government should retain ownership of uranium enrichment facilities, the panel recommends, thereby reversing the Ford Administration's goal of turning enrichment plants over to private industry. The United States should also ensure that there is ample enrichment capacity in the 1980s and 1990s to meet international commitments, for the panel suggests that an assured fuel supply would reduce the incentive for other countries to develop their own enrichment and recycling facilities.

Finally, the panel recommends that the government should proceed promptly with plans to test the feasibility of disposing of nuclear wastes in salt beds and other stable geologic formations. Decisions on waste disposal have been continually deferred, pending introduction of reprocessing, a fact which has led to the widespread belief that reprocessing is an essential pre-requisite for permanent disposal (a belief which is written into legislation in Germany requiring that reactor wastes be reprocessed before disposal). The panel notes, however, that "spent fuel can be disposed of directly, and probably at costs comparable to those for reprocessed wastes". ☐

SERI site selected

The Energy Research and Development Administration (ERDA) last week conferred a much sought after plum on the State of Colorado. It selected a site in the Denver suburbs for the long-awaited Solar Energy Research Institute (SERI), a facility which could develop into the leading solar energy research centre in the United States.

Altogether nineteen organisations in 16 different states had been vying for SERI during the past year; the winner is the Midwest Research Institute, a St Louis-based research organsiation, which teamed with Colorado to offer a proposal.

To help soften the blow for some of the unlucky contenders, ERDA also announced last week that it will establish three regional solar energy research centres in New England, the Southeast, and the upper Midwest. The regional centres are expected to be run by consortia including representatives of several state governments, though their full role in the overall solar effort has yet to be determined.

SERI was called for in the Solar Energy Research, Development and Demonstration Act, a bill passed in 1974 which directed ERDA to establish a national facility and to seek the advice of the National Academy of Sciences on its role and scope. It was generally anticipated that SERI would be a large facility along the line of ERDA's nuclear laboratories.

The Academy furthered that expectation with a report recommending that SERI should be a major operation responsible for all phases of solar energy research including photovoltaics, fuels from biomass, wind power and so on. SERI should have a large budget, amounting to about $50 million by 1980, the Academy suggested. With very few large scientific projects up for grabs these days, such a big-league enterprise immediately generated a lot of attention and proposals came flooding into ERDA.

Early last year, however, ERDA, under pressure from the Office of Management and Budget, opted for something rather less grandiose. It announced that SERI would start small—a budget of about $5 million for its first year—and a decision would be made early in the 1980s on whether it should be expanded to the sort of facility the Academy had in mind. Even that scaled-down version attracted 20 separate bids, however, and ERDA shortlisted 19 of them for further study.

In announcing the selection of the Midwest Research Institute/Colorado proposal, ERDA Acting Administrator Robert Fri said last week that SERI will start with a budget of $4–6 million for its first year, and a staff of about 75 professionals. It will cover all aspects of solar energy, but initially its functions will be mostly concerned with assessments, analyses, information dissemination, education and consultation. Its research and development activities will build up more slowly, and ultimately it could be moved to its own 300-acre site nearby at the foot of the Rocky Mountains.

Colin Norman

BRITAIN

● When the UK Department of Energy (DEN) published the report of its Harwell-based Energy Technology Support Unit (ETSU) on solar energy in the United Kingdom last month, it simultaneously announced the launching of a £3.6 million programme of research and development over the next four years. Full details of how the money would be allocated were not published, but now the government has given a breakdown by department of the overall annual expenditure in the various fields of solar R&D (see table, below).

The details emerged last week at a solar energy workshop organised by the government's Inter-Departmental Steering Committee on Solar Energy R&D; it was attended by some 200 people from national and local government, public and private industry, research institutes and corporations, and universities and polytechnics.

Britain's solar energy effort involves expenditure of some £6 million over four years and stretches beyond the DEN programme, which mainly involves supporting commercial exploitation of solar water and space heating technologies and £600,000 of backing for biological conversion, in which NERC, ARC and MAFF have all shown interest. ETSU meanwhile is to establish this year a register of current R&D on new energy sources and energy conservation relevant to the DEN programmes, but to preserve confidentiality the register will not be published.

The Department of the Environment (DOE) work focuses principally on solar water and space heating too, mainly through its Building Research Establishment. The Department of Industry (DOI) is involved in photovoltaic conversion and supports a solar cell group at the Royal Aircraft Establishment's Space Department, a representative from which last week urged greater cooordination of support from the SRC and DOI.

The DOI now has an information officer attached to the Solar Energy Unit based at University College, Cardiff. As for the attachment of an Energy Research Support Unit to the SRC's Rutherford Laboratory, to provide assistance and support for energy research in universities and polytechnics, this was welcomed by the outgoing chairman of the UK section of the International Solar Energy Society (UK-ISES), who strongly urged devotion by universities to development now that scientific principles were known.

Britain's international involvement is mainly through the EEC, through which international collaboration is being sought in the particular field of thermal power generation, although last week one official from the DOE's Directorate of Research Requirements personally questioned whether UK efforts in this area ought to be concentrated on large-scale plants instead of "something smaller to meet export opportunities".

EEC expenditure amounts to some £320,000 a year in direct support of work at its Joint Research Centre, and £1.75 million in indirect partial support of projects elsewhere.

● Predictably, the first British response to the saccharin developments in North America was a parliamentary question in the House of Commons. The Ministry of Agriculture, Fisheries and Food sees no reason why Britain should ban the sweetener as well, but the data and results of the research are to be examined by expert committees before any action is taken.

The stir caused by the saccharin revelations added poignancy to the publication last week of *Why Additives? The Safety of Foods*, a booklet devised and edited by the British Nutrition Foundation, which is closely linked with the food industry. Many of the scientists who wrote it are also associated with the industry. Although meant to reassure the big public, the views expressed are not as suspect as many bromides which emanate from a nervous industry since, as the booklet explains, the use of additives in this country is strictly controlled, compounds are constantly tested, and use is based on a permitted list.

UK government involvement in solar R & D (£'000 p.a. on average)[1]

	Solar water and space heating	Insolation data	Photochemistry/ Photobiology	Photovoltaic conversion	**Total**
DEN	700	50	150		900
DOE	125				125
DOI	15[2]			215	230
Met. Office		50			50
SRC	35		90[3]	25	150
Total	875	100	240	240	**1,455**

[1] The figures are a rough indication of present allocations, and give an idea of annual average departmental expenditure on the programme over the next few years.

[2] Includes other aspects of solar R & D, not only heating.

[3] With Agricultural Research Council.

The booklet lists saccharin among additives requiring further evidence on safety and toxicology, but stresses that 80 years of widespread use is a good indication that it is "harmless", particularly as regular users include diabetics who see their doctors frequently. The main message is clear: additives make food safer, prolong storage life, allow bulk manufacture or are added for nutritional reasons.

Among additives meant to please, rather than prevent food poisoning or outrageous cost, are colourings. Man-made colourings have been more closely investigated than nature's chemical colours, but the benefits they offer are often purely aesthetic, though attempts to avoid colouring can be commercially disastrous. A former member of the Government's Food Additive and Contaminants Committee (FACC) suggested last week that colouring matter should not be added to infant foods or fresh fruit, meat or vegetables. The case of infant food is currently under review.

Anxiety also exists about the use of nitrites as antibacterial preservatives in bacon and other meat products. The authors of the booklet suggest that the risk of poisoning from botulism is a much greater hazard than any carcinogenic danger, but only experimental evidence can confirm this.

● The stark features about income and expenditure figures of the Imperial Cancer Research Fund (ICRF) —apart, that is, from their sheer size—are that the income all comes from legacies, subscriptions and donations, and the expenditure is channelled into research. Thus, according to the 1976 report published last week, just 3.3% of expenditure in 1975–76 went on administration, and 2.6% to appeals and publicity. Of the remainder—all £8 million of it—£3.9 million was spent directly on research, £2.8 million augmented the ICRF endowment and £1.3 million went on "extraordinary items".

The ICRF chairman, Professor Sir Eric Scowden, says in his report that "far-reaching changes are imminent" at the Lincoln's Inn Fields laboratories. Michael Stoker, the Director of Research, intends to retire in September 1979, and Renato Dulbecco, the Deputy Director, retires this September; the ICRF Scientific Advisory Committee is considering the future of "these vital appointments".

SWEDEN

Politics and power

Wendy Barnaby reports from Sweden on the latest discussion about energy policy

'PRIME Minister Fälldin is thought to be in difficulties', said a Swedish newspaper recently. Considering the energy situation, that is an understatement. Prime Minster Fälldin may well be in his political death-throes.

Only five months ago, after an election campaign in which he promised to rid the country of nuclear power, his appointment as the head of Sweden's three party non-socialist government was widely interpreted as the beginning of the end for nuclear power here. But his government has taken no concrete steps to stop the building—and, in one case, the loading—of reactors. His sympathisers are becoming disenchanted. Over the next few months, the decisions he will have to take will show where his real interest lies: in stopping nuclear power, or in remaining prime minister.

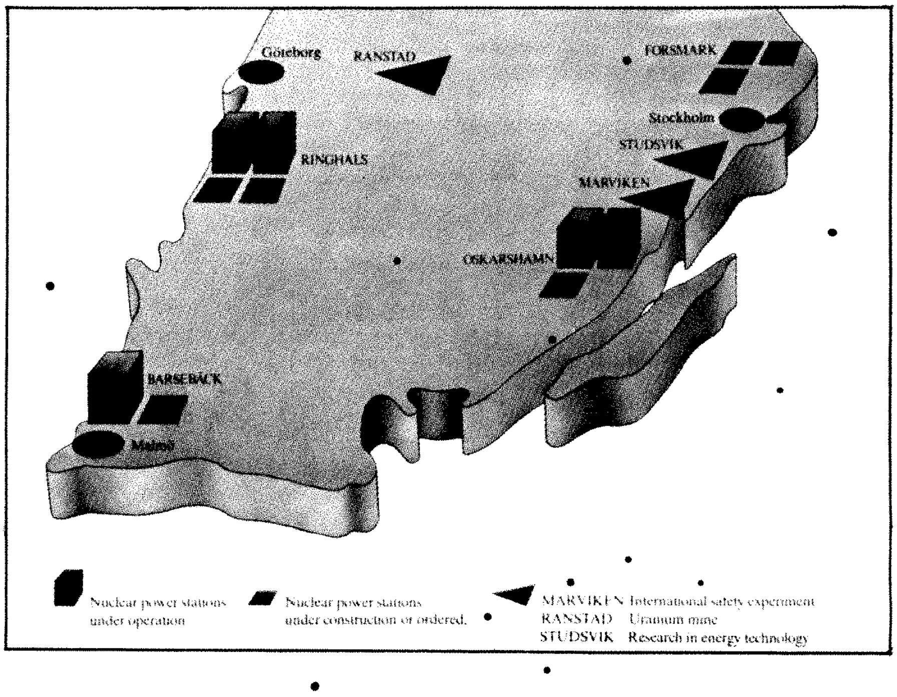
By 31 March the government must advise the Oskarshamn Power Company whether state credit guarantees will be given for the building of the Oskarshamn 3 reactor, now on the drawing board. The decision will also be significant for the owners of the Forsmark 3 reactor, which is at the same stage. The bill, specifying the conditions under which the building of reactors may continue, is expected to be made law in the middle of April. Under its provisions, the owners of any reactor being planned or under construction must present the government with concrete proposals for the 'completely safe' storage of unprocessed fuel or of highly-radioactive waste if the fuel is to be processed: reprocessing agreements must also have been concluded. If these conditions are not met, the government will stop work on the reactor(s) concerned at the end of the year and will compensate owners for costs incurred until the law comes into force in May. Compensation for money spent after May will be subject to special investigation by the government, which has said that it will not pay any bills incurred after it has become obvious that the conditions will not be met. Just exactly when this point is reached will be decided by the government and impartial advisers.

The conditions specified in the bill will have their first test this summer, when the government will have to decide whether or not to allow the loading of Ringhals 3 reactor which is scheduled for 15 July. It will probably be delayed, however, by a legal case brought by environmentalist Björn Gillberg and his lawyer, Gunnar Michelson, who are appealing to the Supreme Court to overrule a decision made last year allowing the release of $175 \text{ m}^3 \text{ s}^{-1}$ of cooling water from the Ringhals 3 and 4 reactors into the sea. There is at the moment neither a reprocessing agreement nor a storage plan for Ringhals 3. In October, the fate of Barsebäck 2, which is already loaded, must be decided—and the loading of Forsmark 1, due to begin next January, will provide the third test under the new law. The main findings of the government's Energy Commission—which is responsible for formulating an energy policy up to 1990 (including a plan for scrapping nuclear power in the mid 1980s)—are expected to be published next March. In the usual Swedish way they will be commented on by all interested parties before the government presents its proposals to parliament late in 1978.

None of these decisions will be easy. The three coalition parties are nowhere near agreed on energy. The Conservatives want 13 reactors by 1985, the Liberals, 11; and both want to retain the government's freedom of action until the Energy Commission's findings are known. The Centre Party would find it impossible to agree to this without finally damning itself as the party which compromised its anti-nuclear faith when faced with the temptations of power. But whatever decision Fälldin takes will bring him enormous problems. If he decides to stick to his election promises, he will be faced with unemployment in the nuclear companies and their sub-contractors, estimated to be as high as 40,000 man-years at a time when industrial unemployment is already worryingly high. If he calls a new election, it is by no means certain that he would win: the latest opinion polls show that the popularity of the Social Democratic opposition is rising. Another option would be to have an advisory referendum; but a special parliamentary bill would have to be passed by majority vote. As the Social Democrats are opposed to a referendum, the government parties would have to unite on the bill; and even that cooperation is in doubt.

Fälldin is confident that the nuclear power companies will not be able to meet the conditions of the new law. Jonas Norrby, Director of the State Power Board—which is building more reactors than anyone else—is just as confident that they will. The nuclear companies are cooperating in their attempts to find 'secure' solutions to the disposal problem, and expect to present a position paper on the subject next September. They are predicting that the political instability will finally put their reactors into operation.

The latest idea for disposal involves encapsulating the waste in ceramic material and then compressing it into a solid harder than granite. This suggestion has come from Asea-Atom, a company which is not building any reactors at all, but which is experienced with high pressure techniques in areas other than nuclear waste. It admits that only time and experimentation will tell whether the idea will work. One of those who doubts that it will is Gösta Wranglen, Professor of Cor-


Göteborg
RANSTAD
FORSMARK
Stockholm
STUDSVIK
RINGHALS
MARVIKEN
OSKARSHAMN
BARSEBÄCK
Malmö
Nuclear power stations under operation
Nuclear power stations under construction or ordered.
MARVIKEN International safety experiment
RANSTAD Uranium mine
STUDSVIK Research in energy technology

rosion Science at the Royal Institute of Technology. He forsees problems arising from the radioactivity of the waste, which would cause the release of oxygen from the water surrounding it. The resulting oxidising environment would not only favour the spread of plutonium but would also oxidise the pyrites in the bedrock into sulphuric acid, making the water extremely corrosive.

Professor Wranglen is doubtful whether any man-made material could withstand such corrosion for thousands of years, but says that, if the waste must be buried, it should be encapsulated in fire-proof steel with a coating of gold about one-tenth of a millimetre thick: thick enough to ensure absolute impenetrability. To avoid the enormous expense of such a method, he suggests that high-level radioactive waste should not be buried at all, but should be stored under supervision in concrete bunkers or drained rock chambers above the ground water table. The heat from the waste would keep the steel cylinders hot and dry for centuries, and they would need no encapsulation at all. He argues that resistance to this solution is purely psychological: acceptance of nuclear reactors themselves implies acceptance that the fuel and waste within them might be used in wars or other catastrophes, and we ought therefore to accept a similar risk in connection with stored waste.

In the face of all these uncertainties, it is hardly surprising that gas as an energy source has become attractive. One of Sweden's ship-building companies, Kockums, recently presented a plan in which the need for nuclear power could be reduced from an estimated ten reactors to six by 1985 if liquified natural gas (LNG) were imported from Arab countries. By 1985, gas would provide 26% of the country's energy needs; dependence on oil would stand at 37% instead of the 47% now expected, and nuclear power would provide 11% instead of an expected 29%.

After the announcement of this proposal, a Swedish-American team, which has been doing research into ways of stopping leaks from underground gas storage chambers, declared that a large ship fully loaded with LNG would have an energy equivalent of a tactical nuclear weapon, and questioned the wisdom of letting such a potential disaster into the Baltic.

Looking further ahead, Sweden may be able to import natural gas from Norway through a pipeline off the projected Norway – Denmark – West Germany line whose parameters are being discussed in Oslo. According to the Norwegians' calculations, gas production would have to be 20 billion cubic metres a year to make the pipeline pay. Present production is only half of this and Norway's Prime Minister, Odvar Nordli, is reported to have refused to guess in which decade the project could begin. All of this can provide only cold comfort for Fälldin: he needs solutions right now. ☐

● Last week, Mr Alexei Kosygin, the Soviet Premier, opened Finland's first nuclear power station—a 440 MW pressurised water reactor—in Loviisa, a small town south of Helsinki. The order for the power station was placed with the Soviet Union in 1970 at a value of £25 million at the then rate of exchange. ☐

PAKISTAN

Bones of contention

Azim Kidwai looks at the place of science in the recent elections in Pakistan

AFTER an exceptionally emotional and at times hurtful election campaign, the ruling Pakistan People's Party led by Mr Zulfiqar Ali Bhutto swept the polls in the elections held for the National Assembly and the Provincial Assemblies on 7 and 10 March respectively. The main opposition, the Pakistan National Alliance, has challenged the results and has refused to accept them on the grounds that the elections were rigged. It boycotted the provincial elections of 10 March saying that the National Assembly elections had been unfair.

Without going into the polemics of the political scene, and accepting that the People's Party is to govern the country for the next five years, certain projections can be made on the emphasis which will be given to science and technology and the future shape of science policy in Pakistan. The policy kept in abeyance while everyone was preoccupied with the elections is now likely to be finalised, but the lessons learned from the elections might distort it to a certain extent; the first priority, one would expect, would be geared to meeting the practical needs of Pakistan as reflected during the campaign.

Science policy as such was not an election issue, and was not accorded any status as a separate issue in the manifesto of either of the major parties. The consequences of scientific and technological advance were nevertheless bones of contention. For example, the unprecedented price-spiralling during the past four years—an almost 100% rise—was the chief subject of debate in almost all election meetings. The National Alliance promised to slash prices by good management, by coming down heavily on the black-market and so on, and some of its leaders said they would bring them down to 1970–71 levels. The people's Party refuted the claim, saying that inflation was a complex international phenomenon and that a developing country, weak in basic science and mainly producing raw materials, had to pay through the nose for finished goods.

According to its manifesto, the People's Party is committed to making Pakistan self-sufficient in grain and oil within the next five years, and to a 50% increase in GNP without neglecting industry and defence. With its victory, therefore, agriculture and oil might take precedence; the polls reflected the greater popularity of the People's Party in the rural areas than in the urban centres (it lost heavily in Karachi, the largest city in Pakistan), probably because it is likely to fund the agricultural sector handsomely.

Agricultural research and inputs like fertilisers, pesticides, new varieties of seeds, and the Irrigation, Drainage and Flood Control Council are likely to be favoured in the development effort. The importance of agriculture in Pakistan is demonstrated by the fact that in 1975–76 it contributed no less than 39,000 million rupees (16 rupees=£1) to a total gross domestic product of 119,000 million rupees. It also happens to be a source of livelihood for almost three quarters of the rural population, which makes up about 70% of the people of Pakistan.

Farm mechanisation could be another priority area. In 1975–76, some 11,000 tractors were imported—a 100% rise over the 1969–70 figure; tillers are to be imported from China and Japan. Productivity in agriculture is still growing very slowly, and is likely to gain impetus through the use of the available scientific and technological techniques.

Luckly for Mr Bhutto, sizeable quantities of oil were found last December at Dhodak in the north. Although the site is primarily a large gas-field, it is estimated to hold about 200 million barrels of oil which can start flowing in the next two or three years. Oil exploration and refineries are foremost on the agenda of the People's Party government. ☐

IN BRIEF

SRC appointment

It is now widely believed that the next chairman of the Science Research Council will be Professor Geoffrey Allen, who will succeed Sir Sam Edwards in October. Professor Allen, 48, is at present at the Department of Chemical Technology, Imperial College, and was until 1975 Professor of Chemical Physics at Manchester University; his interests are largely in polymer science. He has done considerable committee work for SRC, where there seems to be general satisfaction at the prospect of his appointment.

Delaney Amendment review

The recently announced ban on the artificial sweetener saccharin in the United States and Canada has prompted a major review of an uncompromising provision in the food and drug law which forbids the use in food of any substance found to cause cancer in test animals. Known as the Delaney Amendment, the clause has long been a target of attacks by the food industry. It has been staunchly defended by many scientists and consumer groups as a prudent measure to keep carcinogens out of the food supply. Within days of the announcement of the proposed ban, a score of bills calling for repeal of the amendment was introduced into Congress, and last week the Secretary of Health, Education and Welfare, Joseph Califano, announced a review of the Amendment's workings.

RANN resignation

The Director of the National Science Foundation's major programme of Research Applied to National Needs (RANN), Alfred J. Eggers, has asked to be relieved of his duties at the end of June to devote more time to studying problems affecting the growth and health of society. Eggers, who has steered the $70 million RANN programme through a minefield of Congressional criticism in the past few years, offered his resignation in a lugubriously worded memorandum suggesting that "in the largest sense of our future activities with our natural resources in our environment, it may well be that in important respects we are entering a 'zero sum game' ".

Fast breeder wanted

The Highlands and Islands Development Board in Scotland would welcome with open arms the commercial fast breeder reactor (CFR) at Dounreay. At a one-day meeting in Glasgow last week that coincided with the closing-down ceremonies on the Dounreay Fast Reactor (DFR), Professor Kenneth Alexander, who chairs the development board, pointedly observed that the number of employees at Dounreay would rise to 4,500 if the CFR were built there but would sink to 500 if it were not.

Pas de Concorde

THOMAS H. JUKES

A DISPUTE is now raging over permission for landing the Concorde in New York Kennedy Airport. Some of my friends in England and in France tell me that they regard the American prohibition of Concorde landings as an insufferable discrimination. They point to numerous violations of environmental safeguards in the USA, to the concerted din of jetliners taking off in droves from American airports, and to the sonic booms of American military jets. The French say that the American objection to the Concorde is based on the 'PFI' doctrine (pas fait ici).

Against these protestations, I point out to them that the supersonic transport (SST) has become a dominant symbol (and a convenient three letter slogan) to American environmentalists; a tangible epitome of technology run wild beyond the bounds of necessity or justifiability. The noise made by Concorde is said to disturb the mating process of wild birds, a ritual that has taken on an almost sacred significance ever since Julian Huxley published his fascinating observations on the sexual foreplay of Great Northern Divers. Environmentalists point to the Concorde as being wasteful of fuel and as being used only by very wealthy people. The needle-like Concorde is said to threaten the delicate ozone layer, which we have come to regard as being like a fragile soap bubble that envelops our atmosphere, protecting us from skin cancer caused by ultraviolet rays from the sun.

In addition to this injurious effect, however, ultraviolet radiation has a needed role of preventing rickets by its nutritional action. To achieve normal calcium metabolism, most mammals and birds must expose their skins to the sunlight. It is possible to speculate that the spread of the human species to northern latitudes occurred because of a mutation that led to the loss of skin pigment. The pale-skinned mutants could utilise sunlight better for production of vitamin D in their skins than could their darker fellow-human beings. Sunlight was scarce and feeble during the long northern winters, but there was just enough for pale complexioned people and so, we speculate, the whites moved into the temperate and subarctic zones. Once there, they eventually found that the need for direct sunlight by their infants could be alleviated by eating fish liver oils.

But the long era of dependence on sunlight did not really come to an end until a tray of hog millet was pushed under a quartz mercury vapour lamp for ten minutes at the University of Wisconsin by Steenbock and Black. The irradiated millet prevented rickets when added to the diet of rats. In so doing, the millet had served as a 'way station' between ultraviolet radiation and the bodies of animals. From then on, it was no longer necessary to use direct irradiation for preventing rickets in babies. Preformed vitamin D now could be added to food at a trivial cost.

Another evolutionary development, probably a very ancient one, led to the appearance of a biochemical system for combating the carcinogenic effects of ultraviolet radiation: the complex system that removes thymine dimers from DNA, formed by such radiation. The dimers and some adjoining nucleotides in one of the strands are snipped out and removed by enzymes and other enzymes restore the DNA to its original state. Some individuals lack the repair system as a result of a genetic defect, and they are highly susceptible to skin cancer.

We have evolved in concord with our surroundings. Ultraviolet radiation is an example of a stimulus that has a beneficial role at low levels, but is harmful in excess. I am more optimistic for the future of the ozone layer than for the supply of jet fuel.

correspondence

Physical science in Chile

Sir,—My friend and former colleague Alwyn Eades, who recently spent three extremely busy weeks in Santiago, is reported (10 February, page 486) to have said that "it is very easy to be misleading about Chile and very easy to be misled". The truth of this statement is made patently clear by Eades' own article, "Three years after Allende", appearing in the same issue. The article is a mixture of factual information with some personal interpretations and political opinions; it also suffers from some important, and surely involuntary omissions. The unaware reader may find it difficult to distinguish between the first three of these and may not know about the latter; so he may easily be misled by an article which may easily be misleading, especially when the atypical front cover of the issue is politically leading.

Personal interpretations and political opinions are of course entirely subjective and Eades is entitled to make and have his own, but *Nature* is not the proper place to discuss them and I will not do so. I must, however, correct some of his most important omissions and errors lest it be thought that I and/or the Chilean Physical Society share all of his views about what has happened to the physical sciences in Chile.

Eades' account of the development of physical research in Chile is in general accurate. When he writes, however, about the talks for "developing a science policy for Chile" in the early 1970s—which did in fact take place but led to nothing definite—he fails to mention what had been done before and has been done since along these lines. He seems to ignore that the National Commission for Scientific and Technological Development (CONICYT), which was established before Allende in 1968 amongst other things to collaborate in developing such a policy, produced a draft for a National Plan for Scientific and Technological Development in 1974. This draft was carefully studied for several months by a committee in which an important role was played by a sizeable number of leading scientists (including several physicists) who were freely nominated by the universities and by the local Academy of Sciences. The outcome of the committee's work was a much improved version of the plan, which was officially approved by Chile's highest authorities early in 1976. These important omissions may leave the unaware reader of Eades' article under the wrong impression that the development of a science policy for Chile was completely abandoned after Allende and that no official steps had been taken before him.

When Eades writes about what has happened in the physics department of the Engineering School, where I work, he mentions "several waves of sackings for a mixture of political and economic reasons, as a result of which the number of academic staff has dropped from 45 to about 25"; he is wrong. So far there has been a reduction of only one member of staff (and this for economic reasons) and this affected four members of the department's academic staff: one of them had reached the minimum legal age to retire and had to do so and three of the youngest ones were transferred from full-time to half-time positions. The latter three preferred to resign and found jobs in other local institutions. Independently and at a later time, two other people left this department to work in the physics department in the Faculty of Sciences. Over the past three years, a few others have voluntarily taken posts abroad and some who were on study leave abroad chose not to come back. All this has taken the number of academic staff down to its present value of 32.

It is true that four laboratories in this department lie virtually abandoned; but Eades fails to mention that three of these were never in full operation because of the shortage of staff (one of them was a one-man job) and that the fourth was almost abandoned when the Argentinian physicists who formed it were asked to leave the country before Allende's time. Eades also says about one of the Chilean Physical Society's present preoccupations, that "since all elections are prohibited in Chile, it (the Chilean Physical Society) is unable (like many other organisations) to replace those committee members who leave the country or retire", so he thinks that "it may shortly find itself with no committee at all". Elections are forbidden and this was one of our preoccupations while he was here. We have since found, however, that there are legal ways for scientific bodies such as the Societies of Biology, Chemistry, Physics and Mathematics to renew their committees at their own free will and that some had in fact already done so. I am glad to inform him and your readers that we have now replaced one committee member who left the country and another who resigned, and that we are planning on renewing our committee early next year, when most of our membership will gather at the University of Concepción for the next scientific meeting.

Finally, I strongly disagree with Eades' statement that one side-effect of the coup was to set back physics research in Chile by nearly two decades. As his article rightly mentions, twenty years ago there was little or no research in physics in Chile: the first groups were just beginning to form, there was virtually no equipment, the Chilean Physical Society did not exist and the very idea of doing physics research in Chile was thought to be utopian by most. Whatever the results have been of the permanent brain drain from Chile over the past twenty years and the departure of scientists with every change of government, we are now much better off indeed than two decades ago. The large (by Chilean standards) number of scientific papers published in the last three years by Chilean physicists who are still working here is the best proof of this.

Yours faithfully,

Claudio Gonzalez

President,
Sociedad Chilena de Física,
Chile

Not rats, but mice

Sir,—We 'applied biologists' at the Pest Infestation Control Laboratory have been professionally associated with rats and mice for some thirty odd years, in all parts of the globe; I seem to have become our 'coprologer'.

Thomas Jukes (10 February, page 491) has made a slight error: it is not rat faeces that are unscreenable from wheat kernels but mouse faeces. The principle is the same however. I agree with his point about 'filth' in food—it is nearly always purely an aesthetic objection sustainable in the 'developed' world—provided the food has been cooked!

It is a pity therefore that he didn't get that point accurately stated.

Yours faithfully,

R. A. Davis

Pest Infestation Control Laboratory,
Surbiton, Surrey, UK

news and views

The inconstant Sun

from David W. Hughes

UNTIL recently astronomers were convinced that the Sun was a regular, well behaved star of considerable constancy. Its present day behaviour was taken to be typical of its activity over a much longer time span. This attitude is now rapidly changing thanks in no small part to the work of John Eddy of the High Altitude Observatory, Boulder, Colorado. He found (*Science* **192,** 1189; 1976) that there was little or no historical evidence to indicate that the 11-yr cycle of solar magnetic and sunspot activity existed before about AD 1700 and concluded (in *Physics of Solar Planetary Environments* (ed. Williams, D. J.) **2,** 958; 1976) that there seems to be a much more gross change occurring in the Sun's behaviour and especially in the solar constant (the energy flux received from the Sun at the Earth's orbit) over periods of hundreds and thousands of years. These changes do not seem to be periodic.

Maunder minimum

The inconstancy of the sunspot cycle was first noticed by F. W. G Spörer and E. W. Maunder in the 1890s. Spörer was studying the distribution of sunspots with latitude and found evidence that the number of spots in the northern and southern hemispheres of the Sun was not always balanced. To check this he consulted historical records of solar activity and found to his amazement that over a 70-yr period between 1645 and 1715 practically no sunspots were seen. This period has been named the Maunder minimum. The few spots that did occur were invariably at low solar latitudes and were single; only one group was seen between 1645 and 1705. Between 1672 and 1704 there were no northern hemisphere spots at all, in fact there were fewer spots between 1645 and 1715 than in a single active year under normal conditions. Eddy re-examined the historical data (*Science op. cit.*) bearing in mind that 'absence of evidence is not evidence of absence'. He also posed such questions as 'how good were the 17th century observers and their observing techniques' and 'how constant a watch was kept?'.

Most solar telescopes of those days had focal lengths of 2 to 4 m and apertures of 5 to 10 cm, the solar image being projected onto a white screen placed behind the eyepiece. The solar image was large enough to make spot umbrae and penumbrae easily discernable and observers recorded details of white-light faculae, penumbrae filaments, satellite sunspots and most of the observational details known of sunspots today. Several transits of Venus and Mercury were observed during the 17th century as well as some 53 solar eclipses. The fact that the first division in Saturn's rings was discovered in 1675 and that five of its satellites were discovered between 1655 and 1684 attest to the fact that a resolution of 1 arc s could be obtained and that 11th magnitude satellites less than 40 arc s from the limb of a bright planet could be observed. Eddy concluded that spots would have been seen if they were there, a conclusion supported by the fact that the few spots that did occur motivated the discoverers to report their observations immediately in the journals of the time.

Moving from sunspots to phenomena caused by and modified by solar activity Eddy verified Clerke's observation that 1645 to 1715 was a period characterised by a marked absence of aurorae and was also a time of profound magnetic calm. The number of aurorae reported increased dramatically after 1716. Even though the scientific renaissance came to auroral latitudes at that time and Halley's paper in 1716 put the auroral

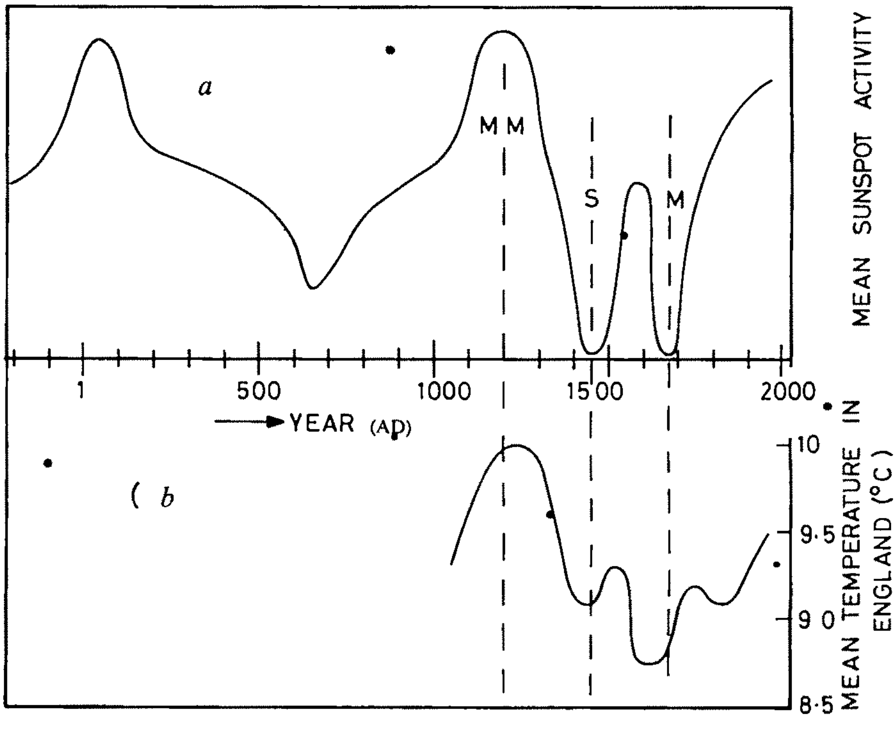


Fig. 1 *a,* A long term envelope over the solar sunspot activity using ^{14}C activity as a guide. MM is the Mediaeval maximum, S the Spörer minimum and M the Maunder minimum (after Eddy in *Physics of Solar Planetary Environments* (ed. Williams, D J.) **2,** 958; 1976). *b,* Estimate of the mean annual temperature in England (after Lamb, *Climate, Present and Past and Future* **1,** Methuen, 1972).

phenomenon on a firm scientific footing, both of these resulting in an upsurge of interest, the 'Maunder minimum' of sunspot activity is evident from auroral activity alone.

In addition it cannot just be a curious coincidence that the chromosphere was discovered in 1706 and that the first description of a structured corona was given in 1715. Coronal shape varies with solar activity. With many underlying spots the corona is large and made up of numerous long tapering streamers extending outwards like flower petals. At solar minimum, with very few sunspots, the corona becomes compressed and bland, the only unusual features being the long symmetrical extensions along the solar equator, this being the zodiacal light (or F corona). Between 1645 and 1715 the corona at eclipses was always described as being limited in extent (1 to 3 arc min above the limb) and as being dull or mournful, and reddish, typical of an extended minimum of solar activity.

Radiocarbon data

Now sunspot data only takes us back with any reliability to the early 17th century. Radiocarbon data on the other hand can extend our knowledge of solar activity back to about 5000 BC, almost half way to the last glaciation. ^{14}C is produced in the Earth's upper atmosphere by galactic cosmic ray bombardment. Through the intermediary of the interplanetary magnetic field the Earth can be shielded from these galactic cosmic rays, high solar activity increasing the field, reducing the cosmic ray flux and lowering the ^{14}C production rate. Trees assimilate ^{14}C as CO_2 in the photosynthesis process so individual tree rings preserve a record of the atmospheric ^{14}C/^{12}C ratio at the time of formation. Figure 1*a* shows the overall envelope of sunspot activity obtained when the ^{14}C concentration is used as an indicator. The Maunder minimum can be clearly seen as can the Spörer minimum (1400–1510) and the mediaeval maximum of activity which occurred between 1120 and 1280. At the present we are half way up an increasing branch of the curve moving towards a time of high solar activity. Figure 1 also shows how these massive solar changes affect terrestrial climate. The lower curve (Fig. 1*b*) shows mean annual temperature in England and it can be seen how the Maunder and Spörer minima correspond to the two coldest extremes of the Little Ice Age when global temperatures were depressed by 0.5 °C to 1 °C.

Solar variability

Eddy is convinced that the individual ups and downs of the 11-yr cycle and also shorter term solar variability are almost wholly unrelated to terrestrial meteorology, this in fact being governed by the total radiative output from the Sun (which is proportional to the solar constant). Öpik (*Irish Astr. J.* **8**, 153, 1968) found that the average value of the measured solar constant had increased steadily, by about 0.28% during the 30-yr period between 1921 and 1952. Figure 1*a* thus also represents the 1% peak-to-peak amplitude variation of the solar constant. Sunspot production is thought to be directly proportional to the rate of radiation flow through the solar photosphere.

In a recent paper in *Solar Physics* (**46**, 3; 1976) Eddy, Gilman and Trotter found that the equatorial rotation rate of the solar photosphere just before the Maunder minimum was 3 to 4% higher than its present value. The rate of change of rotation rate between 0 and ±20° solar latitude was also enhanced by a factor of 3. These results came from an analysis of a series of daily full-disk drawings of the Sun made by Johannes Hevelius between 1642 and 1644 and published in his book *Selenographia Sive Lunae Descriptio* This anomalous rotation may be just beyond the velocity range capable of producing sunspots by the solar dynamo action so the high velocity in the early 1640s could have caused the sunspot dearth that followed.

Dynamo theories of solar magnetic activity rely on the stretching of the general magnetic field of the Sun by a radial gradient of angular velocity in the subsurface convection zone. To explain the migration of sunspots towards the solar equator as the solar cycle progresses this angular velocity must increase with depth below the photosphere. Redistribution of angular momentum throughout the convection zone caused by a variation in the radiation flux and resulting in a decrease in angular velocity deep in the zone could reduce the strength of the dynamo action and switch off the spots.

Tidal influence unlikely

It has often been suggested that the 11, 78 and 179-yr cycles of solar activity are controlled by gravitational tides raised on the Sun by the planets, even though this tidal force is miniscule compared with the force of solar gravity (which is about 10^{11} times larger). Proponents of the tidal hypothesis tend to consider the tides as triggers but as Charles Smythe and John Eddy point out on page 434 of this issue of *Nature* trigger mechanisms have the disadvantage of being out of the reach of physical criticism and usually are beyond the grasp of observational tests. Tidal theories also produce regular sunspot periodicities so how can the Maunder minimum be produced? Planets surely did not stop exerting tidal influence when the spots disappeared.

Using a computer Smythe and Eddy calculate all the two, three and four planet conjunctions that occur between AD 1575 and 2000 and compare their occurrence times with solar activity cycles. There are no dissimilarities between the conjunction frequencies that occur within the Maunder minimum period (1645–1715) and the ones which occur at adjacent periods. They go on to calculate the daily tidal potential at the solar equator exerted by the planets. The power spectrum of this potential, over a 50-yr period, has principal peaks at *J*, *J*/2 and *J*/3 (*J* being the Jovian orbital period of 11.86 yr). The authors find that the power in each of these peaks has not changed perceptibly in the past 500 yr, the power spectra for 1925–1975 being indistinguishable from the Maunder minimum spectra. Tidal theories of sunspot formation thus become much more difficult to support.

So we are presented with an inconstant Sun with sunspots which can be switched on and off in about 10 yr and can stay off for around 70 to 160 yr. The spottiness seems to be tied directly to the total energy flux from the Sun and this seems to vary with time by about 1%. Why it varies and how it directly affects spot production are two questions still to be answered. ☐

H–2 and mating preferences

from J.C. Howard

It ill becomes an immunologist to draw attention to the work I want briefly to discuss. Unhappily, the paper appeared in a journal specialising at the moment in the advancing front of immunology, and was probably rather little read by most of the people best qualified to enjoy it. The paper, entitled 'Control of mating preferences in mice by genes of the major histocompatibility complex' (Yamazeki *et al. J. exp. Med.* **144**, 1324; 1976) reported that, given a choice, a male mouse will tend to pick a female mouse to impregnate on the basis of the alleles she carries in her major histocompatibility (H-2) system. Furthermore the preference shown by the male is itself a function of his H-2 alleles. The preference is not necessarily for the self H-2 type; in some matings it is, for instance BALB.B(H-2^b)♂ chose H-2^b♀

in 70% of trials, in preference to BALB (H-2^d)♀, but in another cross B6♂ (H-2^b) showed a preference for B6-H-2^k♀, and B6-H-2^k♂ for B6♀, rather than for the partner of their own H-2 type. The experimenters used H-2 congenic mice to avoid confusing influences from the genetic background.

This very strange and remarkable phenomenon was appropriately discovered at the Sloan-Kettering Memorial Cancer Center since it was the Sloan-Kettering's President, Lewis Thomas, who first suggested at the second International Congress of Immunology in Brighton in 1974, that the diversity of histocompatibility antigens might be used by animals to identify each other, presumably by smell, thus drawing together two of his persistent interests, immunology and pheromones.

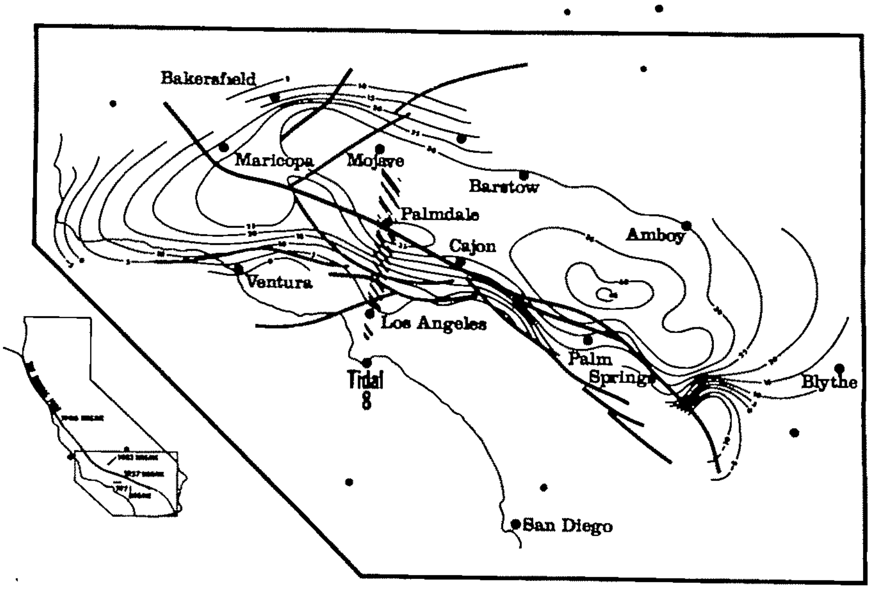
There are many aspects of these findings that need further clarification: do the females do any choosing, or do they merely wait to be chosen? Is the stimulus indeed a smell, and if so from where (the urine?), and precisely what is smelly? Histocompatibility antigens are not obvious candidates for airborne pheromones: perhaps the association with H-2 is indirect, through metabolites from characteristic bacterial flora infesting the skin, or intestinal and genitourinary tracts, the presence of particular organisms being under the control of H-2 linked Ir genes. In this context it was reported a few years ago that the bacterial flora of the human skin was indeed individual, as assessed by characteristic patterns of phage sensitivity, and of course the individuality of human odour has long been known, at least to dogs.

The biological significance of these complex phenomena is not clear. As Thomas and others have pointed out, the recognition of individuals is important for animals with a developed social structure. The H-2 associated mating preferences demonstrated by Lewis Thomas and his colleagues have two additional interesting consequences. In so far as the mating preferences are disassortative they will encourage the persistence of H-2 polymorphism. For a long time, evolutionarily inclined imunologists (and indeed immunologically inclined evolutionists such as J. B. S. Haldane) have been looking for possible 'external' reasons why H-2 polymorphism should be so extensive and so persistent. Until very recently there were no good clues. For a time the effect of H-2 incompatibility on placental weight in mice was offered as a mechanism for the maintenance of the polymorphism: foetuses H-2 incompatible with their mothers had larger placentas and a heavier birth weight than H-2 compatible foetuses in the same pregnancy. There were difficulties in repeating the work, and it has now dropped out of fashion. Furthermore its universal significance as a mechanism for maintaining polymorphism seemed doubtful when it was realised that it was hardly applicable to the birds!

More recently Doherty and Zinkernagel provided a simple mechanism for heterozygous advantage in H-2 when they showed that viral antigens on cell surfaces were recognised in association with H-2 specificities. The virus-infected H-2 heterozygote is thus able to display a more varied antigenic stimulus to itself than the homozygote, with a

Palmdale bulge changes shape

from Peter J. Smith



It is just about a year since Castle *et al.* (*Science* **192**, 251; 1976) reported their discovery of the crustal bulge centred near the town of Palmdale about 65 km north of Los Angeles. This uplift, which apparently began about 1960, had by 1974 reached more than 0.35 m at its highest point and was observable over an area of at least 12,000 km^2 of southern California. Interest in the bulge was enhanced by the fact that Palmdale lies almost on the San Andreas fault and by the discovery that the uplift contours were elongated along the fault over a distance of more than 150 km. Moreover, most of the area with an uplift greater than 0.15 m had remained virtually aseismic since at least 1932. The possibility that the bulge might herald a major earthquake therefore had to be taken seriously.

No such earthquake has yet occurred, but a new analysis of historical and recently acquired geodetic data, announced this month by the US Geological Survey, reveals that the uplift covers a much wider area than previously supposed and that part of it has collapsed since 1974 The zone originally studied by Castle and his colleagues lies between Maricopa and Cajon (see map), but it is now evident that uplift extends at least as far as Blythe in the southeast. The bulge thus stretches for at least 600 km along the San Andreas fault and covers an area of more than 90,000 km^2. Moreover, there is a second peak of uplift (between Palm Springs and Amboy) which is actually greater than the Palmdale peak in 1974.

Since 1974, however, the uplift in the vicinity of Palmdale itself has been reduced by about 0.18 m. The significance of this depression is unclear, although the area has seen similar behaviour before. A less well documented uplift of possibly as much as 0.30 m took place sometime between 1897 and 1914 but had almost disappeared by 1926, which has led Castle to speculate that "the recent deformation may be but a part of a generally cyclic process".

It may be significant that no major earthquake can be associated with the earlier episode of uplift and collapse, which would seem to imply that the present deformation does not necessarily indicate an imminent earthquake. On the other hand, uplift definitely preceded the 1961 Niigata (Japan), the 1971 San Fernando (California) and the 1975 Haicheng (China) earthquakes, among others. The 'Palmdale bulge' may or may not pose a problem to the residents of southern California, therefore. The only thing to do for the time being is to continue to observe it as closely as possible.

correspondingly larger protective immune response. This is the kind of effect that not only guarantees the persistence of H-2 polymorphism (though not necessarily its variety) but also may explain why the mouse, at least, goes to such lengths to increase H-2 heterozygotes in the population above the 'natural' equilibrium frequency, by using recombination inhibition from the neighbouring T system in combination with a high frequency of lethal recessive alleles. Disassortative mating preference by external identification of H-2 products or their effects will operate in the same direction.

Recognition of genetically endowed polymorphic pheromones may also play a part in maintaining the genetic integrity of incipient species in the face of a constant risk of interbreeding with the parent species. John Godfrey has shown for example, that subspecific variants of the bank vole *Clethrionomys glareolus* show a pheromone-dependent assortative mating preference for their own subspecies, given a choice. Some behavioural isolation is clearly a prerequisite for fixation of the subspecific gene complex: it would be interesting if the accumulation in a subspecies of polymorphic variants at genetic systems normally recognised as antigens were to play a part in this important phenomenon.

Clearly the Sloan-Kettering findings cannot be taken lightly. It is therefore a pity that the H-2 associated mating preferences shown by the use of congenic lines were not confirmed in breeding tests, since trivial and unrecognised differences between lines, for example in housing, handling and maintenance could possibly have caused the effects that were seen. I hope that the work is soon confirmed and extended, and that the study of major histocompatibility polymorphisms has just begun a new life far from the inward mysteries of the immune response. ☐

Landslides in sensitive soils

from I. J. Smalley

THE property of many sensitive clay soils in eastern Canada to transform suddenly and unexpectedly to material of virtually zero strength makes this region particularly prone to large landslides. Since the turn of the century, landslides in eastern Canada have cost more than 100 lives and involved the loss of more than 20,000 acres of upland. Another large landslide may threaten this spring on the calculation that these massive earthflows occur on average every four or five years and the last one, in which 31 people were killed, occurred at St Jean Vianney in 1971. 'Sensitive' soils have a very high ratio of undisturbed strength to remoulded strength but the nature of the transition and the factors which control it are not yet established despite the investigations which have followed in the wake of the St Jean Vianney disaster.

The Terrain Sciences Division of the Geological Survey of Canada has carried out extensive mapping in sensitive soil regions, and the geological setting of the problem in eastern Canada is becoming clearer (Gadd, *Proc. 4th Guelph Geomorphology Symp.* 137; 1975). Extensive studies of the pore water chemistry (Torrance *Can. Geotech. J.* **12,** 326; 1975) suggest that the leaching effect first described by I. T. Rosenqvist in the late 1940s should still be considered a significant factor in Canadian clays, although there appears to be no direct correlation between leaching and sensitivity. Another interesting lack of correlation is that between sensitivity and size of landslide; a conclusion reached by Mitchell and Markell after a study of 75 landslides (*Can. Geotech. J.* **11,** 11; 1974).

Interparticle cementation, by producing strong but brittle bonds, could be an important factor contributing to sensitivity Attention has been focused recently by R. N. Yong and his collaborators at McGill University on the presence and importance of amorphous matter as a cementing agent in these clays and it seems that some soils may have an amorphous matter content as high as 40%. Mineralogical studies on sensitive soils still present problems, mainly those of making quantitative estimates of constituents. Some initial thermogravimetric studies have been made on clay from the St Jean Vianney slide and further studies are in progress on material from the vicinity of another large 1971 event—that at South Nation River. The lack of clay minerals is notable; Gillott (*Can. Mineral.* **10,** 797; 1971) was one of the first to draw attention to the predominance of primary mineral particles (as distinct from clay mineral particles) in the Canadian sensitive soils. The soils, as a result, have a very low plasticity index and the low plasticity facilitates the solid–liquid transition. Cementation provides extra strength but once the interparticle bonds are broken there are insufficient clay minerals to influence the abrupt change in strength properties.

Mitchell and Klugman (*Engng Geol* in the press) in a survey of recent Canadian work report that regional differences in sensitive soils are becoming more apparent although the occurrence of landslides of the characteristic form may be more widespread than has been realised. Critical factors may be revealed when the scope of investigations can be enlarged to include the less accessible regions, in particular Labrador An improved classification of sensitive soils is also required and R. Soderblom at the Swedish Geotechnical Institute has recently taken a significant step in this direction (*SGI Prelim. Rept* **55,** 1974). He is concerned with soils of extreme sensitivity (often called quick-clays, after the Swedish term *kvicklera*) and has distinguished two distinct types. Those which require a considerable energy input to cause the solid–liquid transition he calls 'slow quick clays' and those which transform easily are 'rapid quick clays'. The new parameter of rapidity can be assessed using a standard Casagrande liquid limit device, and should prove very useful when sensitive soil comparisons are being made. ☐

Relapses in malaria

from F. E. G. Cox

RELAPSES, months or even years after an initial infection, are characteristic of malaria and in the quartan disease an individual may experience periodic fevers for the whole of his life. Until the discovery of the exoerythrocytic stages of malaria in the liver in 1948 it was widely assumed that during the remissions the parasites persisted in the blood at subpatant levels and that their spasmodic appearances in larger numbers represented recrudescences rather than true relapses. Relapses proper only occur when the blood is quite free from parasites during the intervals between periods of patency. The discovery of exoerythrocytic stages in the liver immediately suggested that it was there that they sojourned and from time to time flooded the blood with new erythrocytic forms. A number of observations, however, have indicated that this explanation is too simple (Garnham *Protozool. Abstr.* **1,** 1; 1977) and recent work in the Soviet Union and Romania suggests an alternative hypothesis.

It has been suspected for some time that the tertian malaria parasite, *Plasmodium vivax,* exists in two forms, one with a short prepatent period (13–16 d) before parasites appear in the blood following infection from a mosquito and the other with a less clearly defined longer period of 300 d or more (Sergiev & Tiburskaya *Arch. Roumaines Path. exp. Microbiol.* **26,** 475; 1967). The short prepatent form is characteristic of tropical strains and the other is typically found in more temperate regions such as the Soviet Union and North Korea Using a North Korean strain a team of nine coworkers, six British and three Romanian, (Shute *et al. Trans. R. Soc. trop. Med. Hyg.* **70**, 474; 1976) have demonstrated the coexistence of sporozoites capable of inducing long or short prepatent periods. They infected humans with 10, 1×10^2, 1×10^3 or 1×10^5 sporozoites and found that in those given 10 or 1×10^2 the prepatent periods ranged from 262–628 d, in those given 1×10^3 the prepatent period in one was 16 d and in four others between 257–386 d and in seven infected with 1×10^5 the prepatent periods were 13, 15 or 16 d. In parallel experiments using a tropical strain of *P. vivax* all the prepatent periods were short, 12–17 d (Ungureanu *et al. Trans. R. Soc. trop. Med. Hyg* **70**, 482; 1976). These experiments suggest that temperate strains of *P. vivax* consist of at least two genetic types, one with a long and one with a short prepatent period and that in any population of sporozoites there are more long prepatent period forms than short whereas in tropical strains the reverse is probably true. The selection of the two forms has presumably been due to differences in the seasonal availability of suitable mosquitoes for transmission.

It may well be that there are more than two forms of *P. vivax* within any one strain and Tiburskaya and Dukhanina (*Med. Parazit.* **45**, 218; 1976) believe that there may be as many as five in one Russian strain and that the expression of a particular form varies from month to month.

The life cycle of the malaria parasites must now be redescribed. Sporozoites are injected into the host and enter the liver where they may develop rapidly or slowly into multinucleate exoerythrocytic schizonts the products of which invade blood cells. There is no need to postulate a continuous series of exoerythrocytic cycles to account for relapses because these could result from initial infections with sporozoites having prepatent periods of up to several years. Differences in the time taken for different sporozoites to mature into exoerythrocytic stages could account for the irregular relapses characteristic of malaria.

Continuous exoerythrocytic cycles are now no longer thought to occur in any species of human malaria. In *P. vivax* and *P. ovale*, sporozoites with long but variable development are responsible for relapses whereas in *P. falciparum* and *P. malariae* small numbers of parasites persist in the blood after the initial infection and subsequent attacks of malaria result from recrudescences of these and are not true relapses It is now, more than ever before, important to know exactly what species is causing malaria because parasites that occur only in the blood respond to a number of antimalarial drugs whereas those that develop slowly somewhere else in the body do not. ☐

Telecommunications in the '80s

from John R. Forrest

A meeting entitled Telecommunications in the 1980s and After was held at the Royal Society on 10–11 March, 1977. A small exhibition also provided a demonstration of telecommunications hardware currently used or under development.

EXOTIC technology, system planning and social implications were all discussed in a balanced programme. Sir Edward Fennessy (British Post Office) saw an optimistic future for telecommunications on the global scale, with some 1,500 million telephones by the year 2000, this figure being dominated by the now-developing countries. He saw electronic letter and document overnight transmission as a very rich field for development in balancing the loading of telecommunications traffic. This optimism in regard to the almost limitless capabilities of technology was shared by K. Corfield (TEMA Ltd), but he showed concern that Britain should itself have a most modern and internationally-compatible telecommunications system as an incentive for a powerful export industry. By contrast,

Dimeric water molecules

from Graham Richards

IN the heady days when 'polywater' was thought to exist and even to represent a danger to life, theoretical chemists were quick to calculate the stability of water dimers and to risk their reputations by lending weight to credence in the new form of water The bursting of the polywater bubble has not, however, ended interest in the dimeric species $(H_2O)_2$. It was first postulated to exist in order to explain pressure, volume and temperature observations in water vapour and later observed directly in solid rare gas matrices and by mass spectrometric techniques. The dimer, trimer and other small polymers are of significance in the understanding of liquid water itself and in the study of the all-important hydrogen bond which must be involved in the stability of water polymers and indeed in most natural polymers.

The quality and reliability of the most recent theoretical predictions using *ab initio* molecular orbital methods is demonstrated by a new detailed experimental study of the water dimer by molecular beam electrical resonance spectroscopy (Dyke *et al J chem. Phys.* **66**, 498; 1977). Molecular beams of the hydrogen-bonded dimers were generated by expanding water vapour through a pinhole nozzle. The cooling so produced reduces the temperature of the beam so that the thermal energy is insufficient to break the relatively weak hydrogen bond. Radiofrequency and microwave transitions were observed arising from changes in the rotational energy of the rotating dimer. This gives structural information as does the dipole moment which can be interpreted to give the relative orientations of the two H_2O monomer subunits.

The resultant structure is the so-called "*trans*-linear" complex This consists of one water molecule joined to the other by means of a linear hydrogen bond. The covalently-bonded hydrogen of one partner points along a lone pair electron lobe of its mate with a plane of symmetry along the hydrogen bond bisecting the HOH angle of the proton acceptor. The non-hydrogen bonded protons are disposed *trans* to the long bond.

The distance between the two oxygens is longer in the dimer than it is in liquid water, a similar result also being known for $(HF)_2$. Apparently then, when a hydrogen bond is formed, charge transfer occurs in such a way as to enhance the formation of further hydrogen bonds which are strengthened, as is the case in liquid water.

Another striking point is that the geometry of the proton acceptor is nearly tetrahedral in conformity with simple ideas about the disposition of electron lone pairs, despite the fact that calculated electron density maps do not show prominent lone pairs on monomeric water. The results do, on the other hand, support the accepted view that hydrogen bonds have an important covalent component and are not merely electrostatic.

The detailed results of this work answer most of the questions about the water dimer, but many remain about the trimer. Is it an open structure with two hydrogen bonds, or is it a cyclic entity with three? No doubt theoreticians will now feel confident to answer this question long before the difficult experiments can be completed.

Mr. D. Elias (West German Ministry of Telecommunications) was more conservative towards exotic technology, with the exception of techniques like Viewdata, seeing as first priority the full development of conventional telephone service to all members of the public. He also stressed the need to take future system development decisions with careful regard to their effects on employment.

Speaking about integrated circuit technology, D. Roberts (Plessey Ltd) showed that although 1 Mbit of memory on a $1''\times\frac{1}{4}''$ chip was likely very soon, the progress to more and more logic elements on a semiconductor chip was likely to be checked by the low yields and higher failure rates as complexity increased, implying an optimum scale of integration. He pointed to the crucial need for very much greater contact between telecommunications equipment and semiconductor device manufacturers so that highly-structured logic devices such as telecommunications-dedicated microprocessors could be developed.

For telephone exchanges, digital switching and computer supervision through stored program control with its ease of modification to suit continued system development, was seen as essential by D. Leakey (GEC Ltd) and R. Ketchledge (Bell Laboratories, USA). Savings in cost and equipment size would also result, but advances in software were still much needed. Because of the diversity in population settlement size, hybrid systems involving local microprocessor control coupled to centralised computers were felt to be the optimum solution by K. Cattermole (University of Essex). Since optical techniques were likely to be used in transmission, he also saw optical switching as a very fruitful research area for future wide-band systems.

In transmission techniques, the most exciting development was undoubtedly optical fibre communication. Fibre attenuation was now down to a few dB km^{-1} in large volume production. Continued research was needed in fibre jointing, fibre reproducibility, laser characteristics and modulation bandwidth, but no fundamental barriers to progress were seen. The optical link for telephone traffic from Martlesham to Ipswich in the UK would eventually be upgraded to a modulation rate of 560 Mbit s^{-1} from the initial 8 Mbit s^{-1}, providing a full test of possible future systems. Although multi-mode, graded-index fibres had bandwidths up to 1 GHz, A. Gambling (Southampton University) was optimistic over the future for the higher bandwidth single-mode fibres.

The continued growth of submarine cable installation at around 16% each year was predicted by J. Tilley (STC Ltd). This was one area where it was felt that digital transmission would not replace analogue transmission for some time, a further generation of analogue submarine cables with bandwidths up to 150 MHz being likely. The optical fibre submarine cable was, however, suggested as an exciting possibility for the next century.

There was very similar traffic growth rate on mature satellite links, but here transmission would be overwhelmingly digital in future. The long-term development, in the opinion of B. Edelson (COMSAT, USA), was the construction in space, using the space shuttle, of large satellite platforms containing replaceable equipment modules. Antenna beams would be electronically shaped and steered

The lectures on planning for the future were almost entirely concerned with the social effects of a highly-developed telecommunication system, which can on one hand integrate nations and populations, or alternatively insulate them from personal contact. G. B. Thompson (Bell-Northern, Canada), in a highly entertaining lecture, felt that neither extreme would occur, but in his opinion our society did not know how to handle information and ran into trouble when too large a proportion of the population was involved in information processing as opposed to materials processing. The basic difference with information is that you can sell it many times over and you still have it, he commented. He felt that some very careful research was needed before our society could adjust to a highly-developed information transmission system.

The overall feeling of the meeting was that the novel technology required for future telecommunications systems posed little problem, the research and development areas being clearly defined. The real difficulties were in the financial resources required for systems of ever-increasing size and the difficulty of organisation and decision. In the words of R. Ketchledge: " . . . only politics and lack of vision stand in the way of the new era". ☐

A hundred years ago

In an article which lately appeared in Nature (vol. xv., p. 308), I gave an account of certain very remarkable discoveries made by Prof. Kühne, of Heidelberg, which added additional interest to the startling announcement contained in a recent communication made by Prof. Boll, of Rome, to the Berlin Academy, to wit, that the external layer of the retina is, during life, of a purple colour, which disappears at death, but which is, during life, continually being bleached by the action of light.

It is with great surprise that I have heard that the prominence given to Prof. Kühne's researches on the "Vision-purple" in my article in Nature has given some pain to Prof. Boll, who probably feels some disappointment in not having been allowed to remain in sole possession of the promising field of research upon which he had entered.

In the particular case in point it would appear that Prof. Boll re-discovered (and, what is more, *appreciated the full value* of) a fact which had really been observed by some others (Leydig in 1857, and Max Schultze in 1866), but which had certainly not become part and parcel of the common stock of scientific knowledge, viz., that the rods of the retina are red, he observed that under the circumstances of his own experiments the colour faded at death, and arrived at the false conclusion that the colour was a function of the vital condition of the retina. He, however, observed the remarkable action of light in modifying the colour. "During life", he announced in his paper, "the peculiar colour of the retina is continually being destroyed by the light which penetrates the eye. Diffuse daylight causes the purple tint of the retina to pale. The more prolonged, dazzling action of the direct rays of the sun entirely destroys the colour of the retina. In darkness the intense purple colour is again restored. This objective alteration of the peripheral structures of the retina brought about by the rays of light undoubtedly occurs in the act of vision". This was the great discovery which Boll made, and with which his name will ever remain honourably connected. Although, however, he had been in full possession of the facts in the month of June of last year, when he demonstrated them to Professors Du Bois-Reymond and Helmholtz, and only published his paper in November, he did not succeed in making the discoveries with which, justly, the name of Kühne is now associated. Kühne showed that if the retinal purple is usually destroyed at death, the result is attributable to the action of light, persistence of the colour being by no means necessarily connected with the living condition of the retina. In his beautiful and far-seeing discovery of the true function of the retinal epithelium cells as restorers of the vision purple, he was fortunate enough to make a discovery which it would be very bold for any one—even for Prof. Boll—to say he would have made, had time and opportunities been granted In saying that Boll had discovered everything referring to the vision purple, M. Warlemont shows that he has not appreciated the fact that two great discoveries have been made, the second supplementing the first, and actually needed in order that the significance of the first should be appreciated.

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articles

Temperature and duration of the shadow of a recently-arrived lunar boulder

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Natural thermoluminescence retained by soil samples from the shadows of some Apollo 17 boulders and from an adjacent sunlit area has been used to derive the temperature in the shade and the length of time for which the shadow has been cast in one case. This has implications for soil mechanics on the lunar surface.

In the near-perfect lunar vacuum, the absence of gaseous convection, and the highly insulating nature of the powdery surface soil, ensure that there are steep temperature gradients across the boundary of a lunar shadow. It is therefore of interest, for example in studying the condensation of volatiles and even of any pristine water vapours, to try to determine the average temperature in the shade on the lunar surface. We have used the thermoluminescence (TL) properties of soil samples from inside the shadows of some Apollo 17 boulders and from an adjacent sunlit area to make such calculations. Some evidence, based on the freshness of boulder tracks observed by the astronauts, suggested that the boulders had not been in their present position for very long. We have therefore attempted to calculate the length of time for which one of the boulders had cast a shadow on our soil sample.

To preserve the natural TL of the shaded samples as much as possible it is important that they should be kept at low temperature after retrieval[1]. Some of the samples used in the present study were kept refrigerated by the staff of the Lunar Receiving Laboratory, at ~ −20 °C, starting about 3 weeks after the Apollo 17 splashdown on 17 December 1972. Since their receipt by us in England (on 22 March 1974), these samples have been kept in our laboratory at the temperature of either dry ice (−78.5 °C) or liquid nitrogen (−196 °C). This continuous refrigeration has proved beneficial. For comparison, a counterpart of each of the shadowed samples has been kept at room temperature from the beginning: the 'freezer counterpart samples'. The sample from an adjacent sunlit area has also, throughout, been stored at room remperature.

The samples concerned were collected from two locations at the Apollo 17 site. From Station 6 came the 'permanently shadowed' soil sample 76240, collected from about 1 m under the north overhang of boulder no. 4, which measured ~5 × 4 × 3 m. The boulder is believed to have been derived from the North Massif and seems to have been moving downhill on an 11° slope to the south. For comparison, a second sample (76260, the 'sunlit sample'), consisting of a 2 cm deep skim soil, was collected from just outside the shadow. From the second location, Station 2, only one sample (72320) was collected: a surface soil from about 20 cm under an east-west overhang of a 2-m boulder (no. 2) lying in a strewn boulder field at the lower slopes of South Massif. This sample seems, from our investigations, to have been only partially shaded. No sunlit sample from an adjacent area was available in this case. Details of the apparatus used by us for TL measurements may be found in ref. 2.

TL glow from shaded and sunlit samples

Figure 1 shows the striking difference observed in the natural TL glow emitted by the shaded and the sunlit samples. The shaded sample from ~1 m inside the shadow (76240, 22) has by far the most natural glow, followed by the one from ~20 cm inside the shade (72320, 4), with the least preserved glow being in the sunlit sample (76261,25). Moreover, the glow starts at successively and appreciably higher temperatures on going from the shaded to the unshaded samples in the above sequence (namely, ~55, ~110 and ~180 °C, respectively), resulting from thermal drainage of TL from the shallower traps under quite distinct lunar temperature environments.

To investigate the effect of room-temperature storage of the

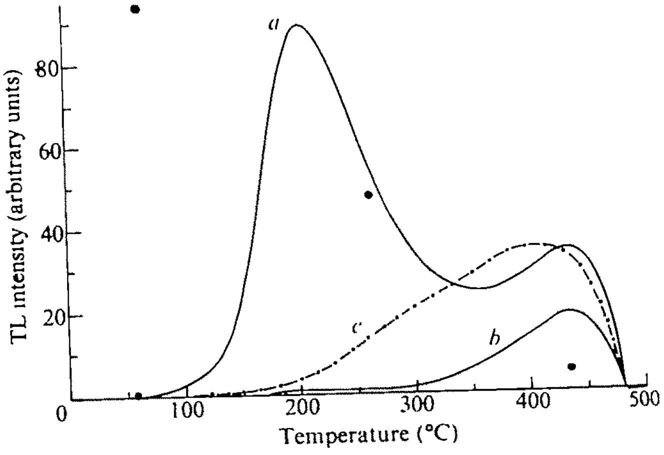


Fig. 1 Natural TL glow curves from the shaded and the unshaded samples. The 'permanently shadowed' sample (76240,22, curve *a*) and the 'partially shadowed' sample (72320,4, curve *c*) have been kept refrigerated (see legend to Fig. 2), while the sunlit sample (76261,25, curve *b*) has been stored at room temperature since receipt on the Earth. Sample weights: *a*, 1.6 mg; *b*, 2.2 mg; *c*, 1.8 mg. For Figs 1–3, the rate of heating in an oxygen-free nitrogen atmosphere is 3.6 °C s^{-1}; sample grain-size d is $45 < d < 106$ μm; the glow is seen by a quartz-window photomultiplier (EMI 6256 SQ) through an Ilford broad-band (375–530 nm) filter no. 622 and plotted an a chart recorder by a sensitive electrometer; black-body radiation has been subtracted from the glow curve.

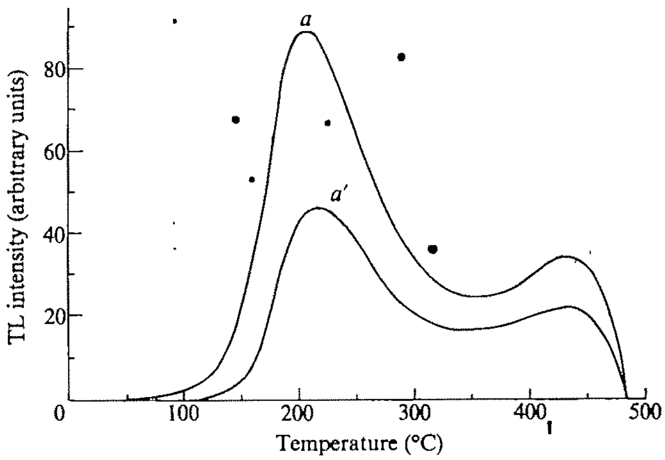


Fig. 2 Fading of the 'freezer counterpart' sample 76241,23 (curve *a'*), kept at room temperature for ~3 yr (date of readout, 26 January 1976), compared to the 'freezer sample' 76240,22 (curve *a*), kept at −20 °C from 11 January 1973–22 March 1974, and at CO_2-snow (−78.5 °C) or liquid-nitrogen (−196 °C) temperatures ever since. Most of the fading appears to be 'anomalous' (that is, not obeying the temperature-dependent equations). Sample weights: *a*, 1.6 mg; *a'*, 1.8 mg.

freezer counterpart samples over ~3 yr between sample retrieval and our experiments, natural-fading and isothermal-annealing studies have been carried out on the samples. Figure 2 shows the natural TL outputs at the beginning of 1976 from the freezer sample (76240) and its room-temperature counterpart (76241). The degree of fading, especially in the high-temperature region, is surprisingly high, suggesting anomalous fading[3]. In Table 1 we record the degree of fading observed in the two counterpart samples 76241 and 72321, after 3 yr of storage at room temperature, by comparing the natural TL surviving in three consecutive readout-temperature intervals of interest to us with the corresponding amounts in the freezer samples 76240 and 72320.

Dose response and inferred natural dose

For various calculations it is necessary to know the natural dose retained by each sample and the characteristics of the growth-to-saturation curves for them. In Fig. 3 we show a typical set of dose–response curves resulting from the artificial irradiation of a sample with ^{60}Co γ rays. The TL intensity from the sample (the shaded sample counterpart 76241, 23, 'sample *a'*') was integrated over three consecutive readout-temperature intervals, 216–306 °C (area I), 306–378 °C (area II), and 378–486 °C (area III), and the resulting 'TL glow areas' are separately plotted. By extrapolating such curves backward, we can infer the natural dose retained in each temperature interval by the sample. Similar curves have been obtained for the sunlit sample 76261, 25 ('sample *b*').

From the growth curves we can also graphically determine the 'half dose' $R_{\frac{1}{2}}$, so termed[2] from analogy with half life in a growth-to-saturation curve in radioactivity. This is the dose required to fill half the remaining traps at any stage of irradiation, assuming the growth law

$$n = N(1-\exp(-0.693\ R/R_{\frac{1}{2}})), \qquad (1)$$

where *n* is the number of filled traps for a total dose *R* (including the natural dose) and *N* is the total number of available traps of a given energy depth.

Theoretical framework

To determine the effective temperature and length of storage of the various samples in their lunar environment, we need to know the trapping parameters *E* ('trap depth', usually in eV) and *s* ('frequency factor', in s^{-1}) of the levels responsible for the observed TL. We have determined these parameters by using the 'initial rise method'[4], and record them and other data needed in our calculations in Table 2.

In our calculations, we have assumed the trap-filling process to obey the 'first-order kinetics' of Randall and Wilkins[5], and followed the general procedure of ref. 2. The following are the essential equations governing the radiation-filling and thermal-emptying of TL traps in generalised environmental conditions.

For a sample with a total of *N* traps, each of depth *E* and frequency factor *s*, irradiated with a dose rate *r* while being held at an absolute temperature *T*, the following differential equation holds

$$dn/dt = -\lambda n + (N-n)rp \qquad (2)$$

where *n* is the number of filled traps at time *t*, λ is the decay constant at temperature *T*, and *p* is the probability per unit dose of filling one of the above traps. For parameters λ and *p*

$$\lambda = \tau^{-1} = 0.693/\tau_{\frac{1}{2}} = s\exp(-E/kT) \qquad (3)$$

where τ and $\tau_{\frac{1}{2}}$ are the mean and the half lives, respectively, of the trapped electrons at temperature *T*, and

$$p = 0.693/R_{\frac{1}{2}} \qquad (4)$$

where $R_{\frac{1}{2}}$ has been defined by equation (1).

The general solution of equation (2) is given by

$$n = \frac{Nrp}{\lambda+rp}\{1-\exp(-(\lambda+rp)t)\} + n_0\exp(-(\lambda+rp)t) \qquad (5)$$

where n_0 is the number of filled traps at time $t = 0$.

There are two cases of special interest to us. First, if all traps are initially empty (for example, when trap-filling first starts in the sunlight), so that $n_0 = 0$, equation (5) is reduced to

$$n = \frac{Nrp}{\lambda+rp}\{1-\exp(-(\lambda+rp)t) \qquad (6)$$

Table 1 Fading of TL in the room-temperature 'counterparts' over a period of 3 yr by comparison with the 'freezer samples'

Sample	Area I (216–306 °C)		Area II (306–378 °C)		Area III (378–486 °C)	
	TL mg^{-1}*	Fading	TL mg^{-1}	Fading	TL mg^{-1}	Fading
76240,22 ('freezer sample')	37.5		15.0		22.5	
76241,23 ('counterpart')†	20.0	47%	7.8	48%	12.8	43%
72320,4 ('freezer sample')	7.2		11.7		18.9	
72321,3 ('counterpart')	4.45	38%	8.3	29%	14.5	24%
76261,25 (sunlit sample)‡	0.68		1.82		7.27	

* TL (integrated over the temperature interval shown in each case) is expressed in arbitrary units, but has been normalised for sample weight.

† Separate studies on the fading of freshly produced TL in sample 76241, kept at room temperature, were carried out to estimate the fading of TL in the 'freezer samples' themselves as a result of the first ~3 weeks of 20 °C storage of all samples at LRL in December 1972. This effect has been ignored in the above table (see ref. 6 for details).

‡ The values for the sunlit sample are recorded for completeness.

Table 2 Values of useful parameters for the shaded sample (*a*) and the sunlit sample (*b*) related to calculations for the temperature and duration of the boulder shadow

Parameter	Sample *a* (76241,23; 'freezer counterpart')			Sample *b* (76261,25)
	TL area I (216–306 °C)	TL area II (306–378 °C)	TL area III (378–486 °C)	TL area III (378–486 °C)
Trap depth E (eV)	1.046 ± 0.05	1.134 ± 0.07	1.439 ± 0.06	1.72 ± 0.06
Frequency factor s (s^{-1})	$\sim 5 \times 10^8$	$\sim 2 \times 10^8$	$\sim 1 \times 10^9$	$\sim 2 \times 10^{11}$
Peak temp. T_p (° C)	279	351	450	441
N/n_{eq} (or N/n)*	7.41	4.58	3.31	3.85
Half-dose $R_{\frac{1}{2}}$ (krad)	500	520	520	340
Storage temp. T_1 (K)	256 (calculated)	256 (from area I)	256 (from area I)	371 (calculated)
Length of storage t_1 (yr)†	—	3.98×10^4 (assuming 48% fading)	5.65×10^4 (assuming 43% fading)	∞
Mean life τ (T_1) (yr)	2.44×10^4	3.50×10^6	7.16×10^{11}	3.85×10^4 (at $T_1 = 371$K)
Mean life τ (20 °C) (yr)	62	5.90×10^3	1.49×10^6	6.48×10^{10}

* These are the values of N/n (uncorrected for fading) as calculated from the heights of the TL dose–response curves (compare Fig. 3) at saturation, and at 0 artificial dose, respectively. In the case of area I (sample *a*) and area III (sample *b*), $n \equiv n_{eq}$ (the corresponding traps having reached dynamic equilibrium in nature).

† These values of t_1 result from equation (11), with $r = 10$ rad yr^{-1}, after a fading correction for the relevant TL area for sample *a* (Table 1) has been applied to the measured N/n value shown in Table 2.

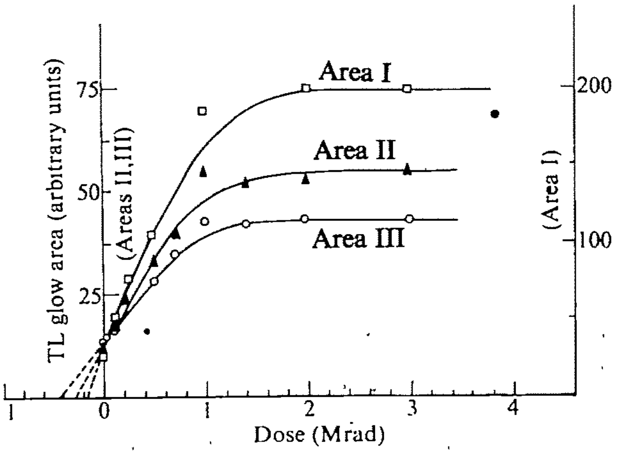
$$\equiv \frac{N}{(1+R_{\frac{1}{2}}/r\tau_{\frac{1}{2}})}\{1-\exp(-0.693rt/R_{\frac{1}{2}})\exp(-0.693t/\tau_{\frac{1}{2}})\} \qquad (6')$$

Second, when the radio-thermal environment changes at time t_0, say, (for example, the boulder arrives), such that the number of filled traps at time $t = t_0$ is n_0, equation (5) holds, except that the values of the relevant parameters are changed to r', p' and λ', and t may be replaced by t_1 such that $t^1 = t - t_0$. Our chief interest, however, is in the case where the time before the arrival of the boulder is very large, so that $t_0 \rightarrow \infty$. The value of n_0 to be used in the modified form of equation (5) is then reduced, by substituting t_0 for t in equation (6) and then setting $t_0 \rightarrow \infty$, to

$$n_0 = Nrp/(\lambda + rp) \qquad (7)$$

The modified form of equation (5), after the arrival of the boulder, thus becomes

Fig. 3 TL response of sample *a* ('freezer counterpart' 76241,23) to γ-ray dose from a ~3,500 Ci ^{60}Co source (dose uncertainty, ±3%). The TL has been integrated in each case over the three consecutive readout intervals termed area I (216–306 °C; □), area II (306–378 °C; ▲) and area III (378–486 °C; ○). Notice the higher scale (at right) for area I From each of the curves the corresponding values of n/N (ratio of the ordinates at artificial dose = 0, and at saturation), and half-dose $R_{\frac{1}{2}}$ (required to fill half the remaining traps at any stage) can be determined. Similar curves were obtained for sample *b* (the sunlit sample 76261,25); figure not shown.



$$n = \frac{N\,r'p'}{\lambda'+r'p'}\{1-\exp(-(\lambda'+r'p')t_1)\} + \frac{N\,rp}{\lambda+rp}\exp(-(\lambda'+r'p')t_1) \qquad (8)$$

A number of interesting limiting cases of equations (6, 6′) exist, details of which is given elsewhere[6]. Here we confine ourselves to two cases of immediate relevance. Thus

(i) for either $rt \gg R_{\frac{1}{2}}$, or $t - \tau_{\frac{1}{2}}$

$$n_{eq} \rightarrow \frac{N}{1+(R_{\frac{1}{2}}/r\tau_{\frac{1}{2}})} \equiv \frac{N}{1+(\lambda/rp)} \qquad (9)$$

and if, in addition $r\tau_{\frac{1}{2}} \gg R_{\frac{1}{2}}$

$$n_{eq} \rightarrow N \qquad (10)$$

where, in equations (9) and (10), n_{eq} represents the dynamic-equilibrium, or steady-state, value of n reached either as a result of giving a massive dose ($rt = R \gg R_{\frac{1}{2}}$) to a sample, or by filling the traps for a very long time t compared to $\tau_{\frac{1}{2}}$ at the temperature concerned; and

(ii) for $\lambda \ll rp$, that is, $r\tau_{\frac{1}{2}} \gg R_{\frac{1}{2}}$

$$n \rightarrow N(1-\exp(-rt\,p)) — N(1-\exp(-0.693rt/R_{\frac{1}{2}})) \qquad (11)$$

The values of N and $R_{\frac{1}{2}}$ in equation (11) are usually obtained by employing such a massive dose rate r in the laboratory that there is virtually no thermal drainage during the irradiation.

Determination of temperature and duration of the boulder shadow

We now determine the effective temperature of storage of the 'permanently shadowed' sample (76240 or 76241, 'sample *a*') and the length of its storage in the boulder shadow, by comparing the TL characteristics of that sample with those of the 'sunlit sample' (76261, 'sample *b*') and making use of the foregoing theoretical framework.

From equation (9), which applies to the case when dynamic equilibrium has been reached in nature (so that $rt \gg R_{\frac{1}{2}}$), we obtain, using equation (3), the value of the prevailing (or 'storage') temperature as

$$T = \frac{E/k}{\ln\left[\dfrac{sR_{\frac{1}{2}}}{0.693r\,(N/n_{eq}-1)}\right]} \qquad (12)$$

where N/n_{eq} is the ratio of the saturation TL (obtained from a separate experiment) to that found in the natural sample. If the natural dose-rate r is known, T can be calculated by determining the values of E, s and R_i for the relevant trapping level. We assume[7] a value of $r = 10$ rad yr^{-1} for unshaded lunar samples. As r occurs in the logarithmic term, even a substantial change (say reduction by a factor of 2) in its value after the arrival of the boulder has little effect on the calculated value of T. This also applies to the effects of fading corrections (discussed below) on N/n_{eq}.

In Table 2 we record the experimental values of all the parameters that are needed in our calculations. Our calculations involve the following steps:

(1) We assume in the case of the sunlit sample (*b*) that dynamic equilibrium has been reached in filling the traps corresponding to the TL readout area III (378–486 °C, with $E = 1.72$ eV), that is, $t_1 \gg \tau_i$ in sunlight. By using the values of the various parameters given in Table 2 (and after applying a 43% fading correction to N/n_{eq} by inference from the corresponding value for sample *a* in Table 1), we obtain from equation (12) the lunar storage temperature $T = 371$ K for the sunlit sample.

(2) We then calculate from equation (12) the storage temperature T_1 in the shade by assuming that equilibrium has been reached in TL area I of the shaded sample (216–306 °C; $E = 1.046$ eV), that is, $t_1 \gg \tau_i$ of the trap at temperature T_1 (compare Table 2). After applying a 47% fading correction to n_{eq} in the 'counterpart' sample, (compare Table 1), we find that the temperature in the boulder shadow is $T_1 = 256$ K.

(3) At $T_1 = 256$ K, the values of τ_i for the traps corresponding to TL area II (306–378 °C; $E = 1.134$ eV) and area III (378–486 °C; $E = 1.439$ eV) of the shaded sample are found to be such that the condition governing equation (11) is fulfilled ($r\tau_i \gg R_i$; compare Table 2). We can therefore solve that equation to obtain the length of storage t_1 in shade from either of these TL areas, using the known values of n/N. This implies that the natural TL in areas II and III of sample *a* is well below the corresponding saturation values.

Assuming the values for the amount of fading observed for TL areas II and III in the 'counterpart' sample *a* (namely 48 and 43%, respectively, compare Table 1), we obtain from equation (11) the following values for the length of storage, t_1, in the boulder shadow:

from area II (sample *a*): 3.98×10^4 yr; from area III: 5.65×10^4 yr.

These values are based on a dose-rate $r = 10$ rad yr^{-1}; if that rate is halved in the shadow of the boulder, the length of storage would be doubled, and so on.

Correction factor for 'anomalous' fading

The calculations based on the foregoing framework are found to be very sensitive to the values of the parameters for the various samples and TL areas that are fed into the equations. We mention in passing that the lengths of storage in the shade, as calculated from TL glow areas II and III of sample *a*, can be made to agree if one assumes an empirical relation for 'anomalous fading'[3], namely: fading $\propto 1/E$, where E is the trap depth. This leads to the following values of anomalous fading in areas I, II and III of sample *a*: 68, 62 and 49%, respectively (and for area III in sample *b*: 41%). If one assumes such fading to have taken place in the shadowed sample since retrieval, the duration of the boulder shadow is obtained (from both areas II and III of sample *a*) as $t_1 = 6.50 \times 10^4$ yr (for $r = 10$ rad yr^{-1}).

Conclusion

That we have been able to calculate the value of the duration of the shadow cast over our sample *a* (namely ~ 4.0–6.5×10^4 yr), and hence the time of the arrival of the boulder at its present location, has important implications for soil mechanics on the surface of the moon. Mitchell *et al.*[8] refer to over 300 tracks "made by boulders rolling, bouncing and skidding down lunar slopes". The Apollo 17 mission provided the first opportunity to study closely these interesting features which were very evident on the Taurus–Littrow hills. The mechanisms for setting the boulders in motion are not well understood, but could include cyclical thermal expansion and contraction, impact cratering and seismic events. Our estimation of the time-scale involved in at least one such event could be helpful in clarifying the picture.

In considering the duration of boulder shadow as calculated from the preserved natural TL in the shaded soil, it could be instructive to consider the role of dust movement on the lunar surface. Criswell[9] postulates the migration of electrically charged micrometre-sized grains from the sunlit areas towards dark or partially illuminated areas. From 'horizon glow' observations made at the time of lunar sunset, Criswell calculates a value of mass-flow rate of $\sim 10^{-8}$ g cm^{-2} yr^{-1} for the surface dust, corresponding to a layer turnover rate of ~ 6 μm yr^{-1}. Such a rate of inflow would deposit a layer ~ 30 cm thick in 50,000 yr under the boulder. That this is not the case is clear when one considers the top ~ 5 cm of the shadowed soil (the maximum depth of our scooped sample *a*). A thickness of 5 cm would, at the above rate, be deposited in $\sim 8{,}000$ yr, yielding an average age of only $\sim 4{,}000$ yr inside the shadow, whereas our calculated length of storage in the shade for sample *a* is 40,000–65,000 yr. Assuming the top 1 cm in the shade to be of external origin, the Criswell rate of inflow must be reduced by a factor of ~ 40. (This results in the duration of the boulder shadow being revised upwards from 6.0×10^4 yr to 6.67×10^4 yr to yield the observed amount of glow, assuming a homogeneous mixing of the top 5 cm of soil during and since its retrieval.)

The value of the storage temperature in the boulder shadow obtained by us (256 K) seems to be high when one considers the conductive processes on the Moon. Values as low as ~ 25–100 K can be obtained by using the lunar heat-flow data of Langseth *et al.*[10] or our own conductivity data[11], but such calculations are strongly model dependent. Our value in the shade is quite close to the mean equilibrium temperature on the lunar surface (~ 240 K). Imperfect shadowing of the sample (which came from only ~ 0.5–1 m inside the boundary of the shadow) and soil mixing could explain the rather high value of the effective storage temperature in the shadow of the boulder estimated by us. Our calculation depends critically on the value n_{eq}/N (the fraction of total traps filled at equilibrium) in the TL glow area I of sample *a*. If, as a result of possible exposure to sunlight during the retrieval procedure and/or the high-temperature ($\gtrsim 100$ °C) handling of the sample during EVA on the lunar surface, or because of a greater degree of anomalous fading than we have allowed for, the initial value n_{eq}/N was higher than that used in our calculations, the temperature in the shade would have to be revised downward. Similarly, if dynamic equilibrium had not as yet been reached in trap-filling for area I of sample *a* (owing, say, to a relatively short storage time in the shade), then equation (12) would not hold, and the value of storage temperature obtained by us would be an overestimate.

Our investigations have, however, been limited to the measurable effects in the context of information currently available, and the values obtained by us are the best estimates that can be made at present for the temperature and duration of the shadow of the lunar boulder concerned. Further refinement of the data may be possible in our future work which involves the simulation of the lunar irradiation conditions.

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Climate periods in tree, ice and tides

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Ten climate periods found in stable isotope ratios of oxygen and hydrogen, measured in 1,800 yr of Japanese cedar rings, agree with climate periods found in 800 yr of Greenland ice and with periods computed from the tidal stresses of the Sun–Moon–Earth system, and with periods found in the ^{14}*C record of the bristlecone pine sequence of southern California. The Greenland oxygen ratios have previously been found to have opposite phase to the* ^{14}*C ratios of the bristlecones, and we have found also an opposite phase between oxygen and hydrogen ratios in the Japanese cedar on the one hand and* ^{14}*C in bristlecones on the other hand.*

VARIATIONS of the oxygen and hydrogen isotope ratios have been measured in a Japanese cedar ring sequence of about 1,800 yr (see Fig. 1 and ref. 1). The Fourier transforms of these isotope ratios against age, into power against reciprocal periodicity, show the 10 periods enumerated in Table 1. Eight of these periods are evidenced in variations of the oxygen isotope ratio in Greenland ice[2]. Three of these 10 periods are evidenced in tidal stresses calculated for the Sun–Moon–Earth system[3]. The periodicities evaluated from these four sets of data agree with each other within statistical accuracy.

Why do the periods lie between 50 and 300 yr? The answer is to be found in the nature of sampling. Our cedar sequence was sampled integrating over 5 yr at a time and therefore cannot reliably show evidence for periods of less than about 50 yr. To look for periods of < 50 yr in a sequence of 5-yr samples or to look for periods of > 300 yr in a sequence of 1,800 yr is probably meaningless. Such periods may appear in Fourier transforms but they do not necessarily represent reality.

To understand this we generated random signals of values 0 or 1, and assigned them to 10-yr intervals in 1,000-yr hypothetical sequences, and made their Fourier transforms. In three such computer experiments we always obtained a period of about 20 yr as an artefact of the sampling interval. In part it occurs because short sequences of random numbers (say 100 numbers) are not really random, and in part because it

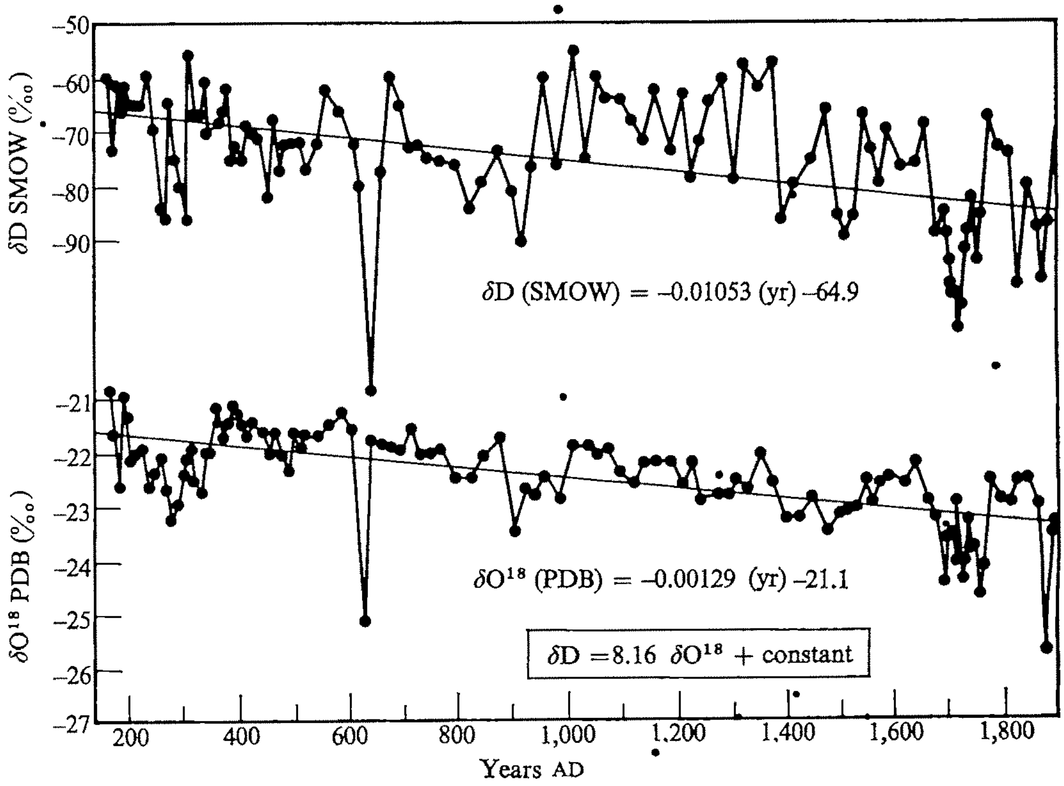

Fig. 1 Stable isotope ratios of oxygen and hydrogen measured in Japanese cedar, AD 140–1950, in whole wood samples, integrating over 5 yr each. PDB, Pee Dee Belemnite, a fossil shell used as standard; SMOW, standard mean ocean water.

Table 1 Periods and respective powers evaluated in Fourier transforms of oxygen and hydrogen istotope ratios in a Japanese cedar AD 140–1950 (ref. 2) and in oxygen isotope ratios in the Greenland ice cap, AD 1200–1950

δ ($^{18}O/^{16}O$)*		δ (D/H)*		δ ($^{18}O/^{16}O$) (ice and tides)†	
Period (yr)	Relative power	Period (yr)	Relative power	Period (yr)	Relative power
54 ± 3	0.58	52 ± 5	1.00	55 (b)	—
57 ± 3	0.65	57 ± 3	0.60	62 (b)	—
59 ± 3	0.70	61 ± 2	0.40	68 (b)	—
69 ± 3	1.50	66 ± 4	1.64	78 (b)(a)	strong
92 ± 2	0.52	87 ± 3	1.00	86 (a)	—
96 ± 2	1.29	95 ± 2	2.18	100 (b)	—
154 ± 6	2.58	153 ± 7	2.18	142 (a)	—
174 ± 7	1.29	176 ± 7	2.00	179 (a) 181(b)	strong
202 ± 7	1.45	205 ± 9	1.70	—	—
271 ± 11	1.80	273 ± 20	1.64	285 (a),(b)	—

* Data from ref. 1.
† Data from ref. 2.
(a) Denotes tidal[3] and (b) denotes ice core[2]. At about the 86-yr period the tidal data and ice core data are almost exactly 180° out of phase.

provides an alias of the sampling interval (see ref. 4 and consider the following example):

Let each sample integrate over $\Delta t = 10$ yr in a sequence of 100 samples corresponding to 1,000 yr. Such a short sequence, even if chosen randomly, produces artefactual signals at periods of 10, 20, 30, . . . yr in the power spectrum, as we have found in computer experiments with random numbers. Then the sampling frequency,

$$f_n = (1/2\ \Delta t) = 1/20\ y = 0.05 \text{ cycles per yr}$$

combines with the artefactual signal frequency,

$$f = 1/20\ y = 0.05 \text{ cycles per yr}$$

to produce the following alias signals,

$$f,\ 2f_n \pm f,\ 4f_n \pm f, \ldots$$

equivalent to

0.05 cycles per s, (0.1 ± 0.05) cycles per s, (0.2 ± 0.05) cycles per s, . . . so that the original signal at 20 y is reinforced by the alias signal at $1/(0.1 - 0.05) = 20\ y$.

If instead, the sampling period is 5 yr, corresponding to $f_N = 1/5 = 0.2$ cycles per yr the artefactual signals lie at $f^{-1} = 5, 10, 15, \ldots$ yr and the aliased signals are all at periods of $\leqslant 10$ yr.

Blackman and Tukey (ref. 4, pp. 31–32) warn us ". . . frequencies above f_N usually contribute some power. Each frequency, no matter how high, is indistinguishable from one in the band from 0 to f_N." Fortunately, there is little power in our D/H and $^{18}O/^{16}O$ spectra at high frequencies so we feel safe from aliasing at periods greater than about 20–30 yr. Thus we are in conformity with the remark (ref. 4, p. 52) that "It is likely that (power) decreases substantially as f goes from 0 to (the sampling frequency). If it does not, then aliasing is likely to have been serious, and satisfactory analysis at this spacing may be impossible." So we seem to be safe from aliasing for periods greater than 50 yr.

If we are guided by ref. 4, p. 11, the maximum oscillation which we should search for in a sequence of 1,800 yr is "a moderate fraction (perhaps 5–10%) of the length of the record" or 90–180 yr, but we shall stretch this to 300 yr because of the agreements among the four sets of periods shown in Table 1. Thus where we are cautioned[4] to study only periods which can oscillate more than 10–20 times in our data sequence, we have dared to take seriously periods which can oscillate 6 times or more in 1,800 yr.

With respect to a DC component, ref. 4, pp. 48–49 states, "All the DC power belongs in the very lowest frequency band . . .". We took the precaution, however, to subtract the DC component from both the D/H and $^{18}O/^{16}O$ data (as in ref. 4, p. 48). Thus as shown in our Fourier transforms[1] there is very little power in periods of more than 400 yr. Furthermore, we have excluded periods of >300 yr from our present considerations because they can oscillate less than 6 times in 1,800 yr.

The ratio of peak height to background in computer experiments on relatively short sequences of random numbers gives a way to assess what is the background caused by 'noisiness' of the isotope data. The peaks listed in Table 1 are significantly above background as shown in ref. 1, Fig. 7.

The validity of a 300-yr period in 1,800 yr of data can be tested by another computer experiment. Specifically one may generate a 300-yr periodic sine function for 1,800 yr, mix it with sine functions of shorter periodicities, and transform the mixture to show that the 300-yr signal is evidenced. Similar computer experiments were performed to see if short periods of 54, 57 and 59 yr are meaningfully separable. Our results indicate that they are. The measurements of Dansgaard *et al.*[2] were made on samples integrating over about 14 yr each, in an ice sequence of about 800 yr.

Thus, are periods between 50 and 300 yr in the ice cap, and even in the tree, representative of real climate variations? The remarkable agreement between the three sets of data suggests that they are. The agreement is expected, and explained, of course, because the ice cap is storing rain water and the tree is likewise storing rain water by changing it chemically to wood.

The evidence that the tree is storing a record of the climatically induced changes in rain water is that the least squares correlation of D/H against $^{18}O/^{16}O$ in the 1,800-yr sequence yields the relation[1]

$$\delta D/H = 8\delta^{18}O/^{16}O + \text{constant.}$$

The empirical slope of 8 is the same as in world-wide rain water within experimental error[5].

Thus the question of whether hydrogen and oxygen isotopes, bound in wood made in previous years, can be exchanging with sap of the current year, seems to be answered conclusively in the negative[1]; specifically, rings of previous years are sealed against exchange. If both O and H in OH groups of wood exchange with sap water, the slope cannot be 8, because there are twice as many H atoms bound to carbon, and these do not exchange.[6]

Fig. 2 Relative phases of ten climate periods evaluated for D/H, plotted against relative phases evaluated for $^{18}O/^{16}O$. Each point is marked by its respective period in yr. Phases are in radians. The dotted line is the best fit straight line, for which alpha $\delta\ ^{18}O = 1.13$ (alpha δ D) -0.771, and $r = 0.922$.

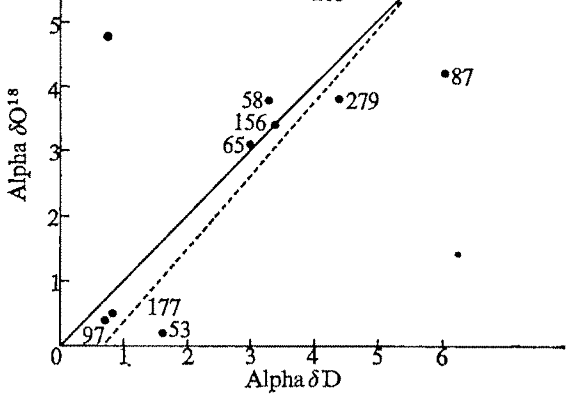

Table 2 Periods and their relative amplitudes and phases

T (period)	A (amplitude)	Alpha (α)	T (period)	A (amplitude)	Alpha (α)
279	3.51	4.40	287	3.29	3.81
205	3.21	4.57	205	3.09	5.46
177	3.71	0.77	179	2.70	0.53
155	3.96	3.37	157	3.96	3.37
97	3.91	0.73	96	2.73	0.39
86	2.42	6.09	88	1.75	4.19
65	3.26	3.02	70	2.90	3.06
62	1.64	6.47	66	1.49	6.21
58	2.06	3.27	57	2.04	3.78
53	2.63	1.56	54	1.84	0.21

Data based on Fourier transforms of our oxygen and hydrogen isotope ratios in a Japanese cedar AD 140–1950.

The remarkable agreement between our tree records of D/H and ^{18}O/^{16}O, and the ice record of ^{18}O/^{16}O, shows itself in yet another way. We have found the D and ^{18}O ratios for the Japanese cedar to be significantly correlated in opposite phase to the ^{14}C variations in the bristlecone pines of Southern California.[7]. Similarly Dansgaard *et al.*[2] found the ^{18}O record in Greenland ice to be significantly correlated in opposite phase with bristlecone ^{14}C.

We have also made a Fourier transform of the bristlecone pine ^{14}C record and find the following periods: 96, 183, 202 and 272 yr, in agreement with the longer periods in Table 1. Periods of <100 yr are probably not meaningful in Suess' measurements[8] because of his 30-yr sampling period.

The Fourier transform also evaluates amplitudes and phases of the periods evidenced in the measurements. For the isotope ratios measured in the Japanese cedar, these are listed in Table 2 for oxygen and hydrogen, and are in agreement with each other. A graph of the hydrogen phases versus the oxygen phases shows this agreement (Fig. 2).

We conclude from these observations—(1) The climate variations in Greenland, southern Japan, and southern California have had the same periodicities for the last 800 yr or more. Climate variability therefore seems to be a global phenomenon. (2) The calculation of Dansgaard *et al.*[2] of the age of ice against depth in the Greenland ice cap seems to be correct, with an error of not more than a couple of years, at least over the last 800 yr. The Japanese cedar rings have been dated by ring counting and by about 50 radiocarbon dates in the 1,800-yr sequence[9]; this serves to calibrate the calculated age of the ice by means of the present comparison of climate periodicities in the tree and in the ice[10].

Our conclusions depend on measurement of hydrogen and oxygen isotope ratios in a single tree: substantiation requires measurement of other tree sequences.

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Comparative neutron small-angle scattering study of small spherical RNA viruses

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Small-angle neutron scattering from solutions of small RNA viruses has been used to study protein–nucleic acid organisation. In the five viruses investigated the RNA is confined to a sphere of about 100 Å radius, with a central hole (with one possible exception). The interpenetration of RNA and protein varies with viruses and seems to be related to the nature of the forces stabilising the virus.

THE basic principles of the architecture of viral protein shells are now well established[1], and the detailed assembly of protein subunits in the capsids of several small RNA viruses has been determined by electron microscopy and X-ray diffraction[2,3]. Very little is known, however, about the nucleic acid. Indeed, electron microscopy provides no information on the internal structure of small viruses, and low-resolution X-ray diffraction does not allow unambiguous location of the RNA, which represents, at most, 35% of the total mass. Klug *et al.*[4] compared low-resolution X-ray diffraction patterns of Turnip Yellow Mosaic Virus (TYMV) virions and the associated empty capsids, and attempted to modify the relative contributions of protein and RNA by increasing the solvent electron density by addition of salt. The contrast variation method has also been used by Harrison[5] and Zipper *et al.*[6]. It is limited, however, because high ionic strengths may dissociate virions or modify their structure, and it is practically impossible to raise the density of the solvent to the level at which it matches that of compact RNA.

Neutron scattering by virus solutions provides a straightforward alternative for obtaining low-resolution information about the viral nucleic acid, and utilises the fact that H_2O–D_2O mixtures can be made with scattering powers that match either RNA or protein[7,8], making it much easier to distinguish between protein and hydrated RNA at low resolution. The possibility that D_2O may affect the stability or the structure of the virus

Table 1

Name	Protein subunit MW	RNA content (MW) of the virion	Total MW
Turnip Yellow Mosaic Virus (TYMV)	20,130	1.9×10^6	5.53×10^6
Southern bean mottle virus (SBMV)	29,390	1.4×10^6	$6 69\times10^6$
Cucumber mosaic virus (CMV)	26,000	$\sim1.10\times10^6$ (multigenome)	5.78×10^6
Bromegrass mosaic virus (BMV)	20,300	$\sim1.05\times10^6$ (multigenome)	4.70×10^6
MS2	13,750	1.142×10^6	3.66×10^6

must be checked in each case, but D_2O is less likely to have an effect than high salt. The method is of general application and allows a simple systematic comparison between viruses. We report here the results of such a survey made on the five viruses, all with triangulation number[1] $T = 3$ (180 subunits), listed in Table 1.

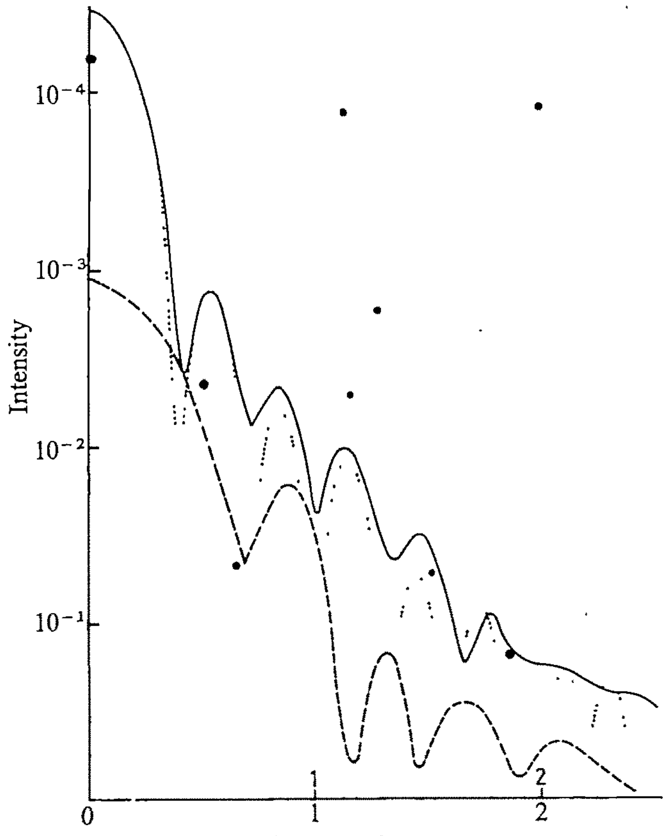


Fig. 1 Typical diffraction curves obtained for SBMV solutions, corrected for background (which is flat). ——, Buffer solution (20 mM phosphate, *p*H 7) in H_2O. ----, Buffer solution with 43% D_2O. The large shift of the first maximum in 43% D_2O shows that the RNA is spread over a much smaller volume than the virus. . . . . ., Curve calculated for the uniform spheres model (Fig. 2).

The viruses were selected to look for a possible correlation between stabilising forces[9] and morphology. TYMV is characterised by very strong protein–protein interactions[10], as shown by the existence *in vivo* of stable empty capsids. CMV, by contrast is stabilised mainly by RNA–protein interactions[11], being sensitive to ribonuclease and to high ionic strength. In bacteriophage MS2 both types of interactions are important[12]. BMV belongs to a special category: at acid *p*H it is insensitive to ribonuclease and high salt and empty shells can be formed *in vitro;* at *p*H 6.5 it swells, becomes ribonuclease sensitive and dissociates in high salt[13]. The physical chemistry of SBMV is not yet well documented, but this virus is under study by X-ray crystallography[14,15]. All viruses were dissolved in low molarity buffers ($\leqslant$ 0.02 M).

Data collection and analysis

The details of the method will be described elsewhere[16]. It consists in collecting the small-angle diffraction pattern of the virus solution at various levels of contrast between virus and solvent, that is, at various solvent D_2O contents. The contrast, $\bar{\rho}$, is defined as the difference between the average scattering density of the virus and the scattering density of the solvent. A typical diffraction curve is shown in Fig. 1. This intensity curve can be put on an absolute scale by comparison with scattering from water and from the intensity scattered at zero angle by a dilute solution of a well-known virus (here TYMV).

A complete analysis of this set of curves gives the following information.

(1) Radius of gyration

For small values of the scattering vector s ($s = \sin\theta/\lambda$ where θ is the scattering angle and λ the neutron wavelength), the scattered intensity varies as

$$I = I(0)\exp\left(-\frac{4}{3}\pi^2 s^2 R_G^2\right)$$

The radius of gyration R_G varies with the mean contrast, $\bar{\rho}$. The relation between R_G and $\bar{\rho}$ is[17]

$$R_G^2 = R_{GV}^2 + \frac{\alpha}{\bar{\rho}} - \frac{\beta}{\bar{\rho}^2}$$

Table 2

Virus	α_{exp}	R_{GV}	α_{cal}	Position of first subsidiary maximum in H_2O	in 43% D_2O	in 68% D_2O	Relative intensity of the first maximum $I(0)/I_1$ in H_2O	in 43% D_2O	in 68% D_2O
TYMV	3,450	111	3068	6.43	8.91	5.79	107	60	28
SBMV	2,840	123	2530	6.27	8.91	5.79	85	16	36
CMV	1,680	127	2160	6.30	8.75	5.79	60	20	34
BMV low *p*H	2,870	113	2455	6.92	9.55	6.16	65	not measured	30
BMV high *p*H	4,720	134	4400	6.06	7.16	5.44	40		26
MS2	4,710	117	5600	6.53	8.59	6.05	46		20

R_{GV} in Å; α is dimensionless, α_{cal} is calculated as explained in the text.
Positions of intensity maxima are given in $\sin\theta/\lambda$ and in units 10^{-3} Å^{-1}.
I_1, the intensity of the first maximum, is expressed by the ratio $I(0)/I_1$, where $I(0)$ is that of the central peak. For a solid sphere the ratio $I(0)/I_1$ is 135; it decreases for a hollow sphere with the size of the central hole, reaching 20 for a thin spherical shell.

Table 3 Results of our survey on five viruses

	R_{ext} (Å)	Protein peak position (Å)	RNA radius (outside) (Å)	Central hole (Å)	Capsid inside radius (Å)	RNA peak position (Å)	RNA peak density (Å^{-2})	Protein peak density (Å^{-2})	ρ protein in H_2O (calculated; Å^{-2})
TYMV	143	117	103	<30	~95–100* 98	~45	2.1×10^{-6}	2.50×10^{-6}	2.47×10^{-6}
SBMV	145	125	100	~30	~80–85 ~100	~55	1.5×10^{-6}	2.01×10^{-6}	2.34
CMV-D	145	117	100	~60	~80–85 ~80	~70	1.6×10^{-6}	2.10×10^{-6}	2.38
BMV low *p*H	130	110	95	~50	~80 ~80				
BMV high *p*H	152		130	~75					
MS2	135	115	100	~60	~105 ~100	~65	1.55×10^{-6}	2.50×10^{-6}	2.40

Capsid inside radius (a) From $I(0)/I_1$. (b) From Fourier transform.
*Data have also been collected on TYMV empty capsids, giving the same results as for TYMV protein shell[16].

A linear variation of R_G^2 with $1/\bar{\rho}$ has been obtained for all the viruses investigated. The values of R_{GV} and α are listed in Table 2; β is zero within experimental error in all cases. R_{GV} is in first approximation the radius of gyration of a homogeneous particle with the same morphology as the virus, but spatial inhomogeneities of H–D exchange also contribute to it[8,17]. A value of $R_{GV}^2 < 3/5\, R_{ext}^2$ (expected for a uniform sphere), where R_{ext} is the outside diameter of the virus (see below), suggests the presence of a central hole, because the radius of gyration of a hollow sphere is larger than that of a solid sphere of the same diameter. α is the second moment of the density fluctuations around their average, at zero contrast between solute and solvent. These fluctuations are determined by the internal structure of the virus. The values of α agree well with those calculated for very simple models consisting of a sphere of RNA surrounded by a protein shell, with dimensions taken from Table 3. β should be zero if the centre of gravity of the scattering mass does not vary with contrast, for example, if the protein shell and the RNA have the same centre of gravity. The experimental data show this to be the case. A separation of 10–20 Å between centres of mass of RNA and protein would not, however, be detectable in our experiments.

(2) The dimensions of the virion and its RNA

The simplest way to analyse small-angle scattering curves from 'spherical' viruses is to find the dimensions of the homogeneous sphere (or hollow sphere) which best fits the innermost part of the diffraction pattern, in particular the position and the intensity of the first subsidiary maximum (Table 2). The non-spherically-symmetric components of the structure do not contribute to that maximum[3] and a first set of values can be obtained by considering the virus to be composed of two spherical shells, each with uniform density, one for protein and one for RNA. Moreover, data have been obtained in 43% D_2O, where the diffraction pattern is dominated by the contribution of RNA, and 68% D_2O, where it is essentially due to protein. The radii obtained are self consistent. For instance the outside diameters deduced from the scattering in H_2O and 68% D_2O are the same, and the sizes of the central hole deduced from the data in H_2O and 43% D_2O. This first set of values can then be used to build models, which are refined using several subsidiary maxima. The values finally obtained (Table 3), are close to the first set. We have already seen that those values account well for the variation of the radii of gyration with contrast. The outside diameter is also confirmed by the Fourier transform of $I(s)$, which equals zero at the maximum diameter of the diffracting mass [18]. The values obtained for the outside diameter agree well with previous results for TYMV (ref. 4), BMV (ref. 20) and MS2 (ref. 6). The large intensities of the first subsidiary maxima (Table 2) strongly suggest the existence of large central holes in all of these viruses, with the possible exception of TYMV, where the hole (if any) is much smaller.

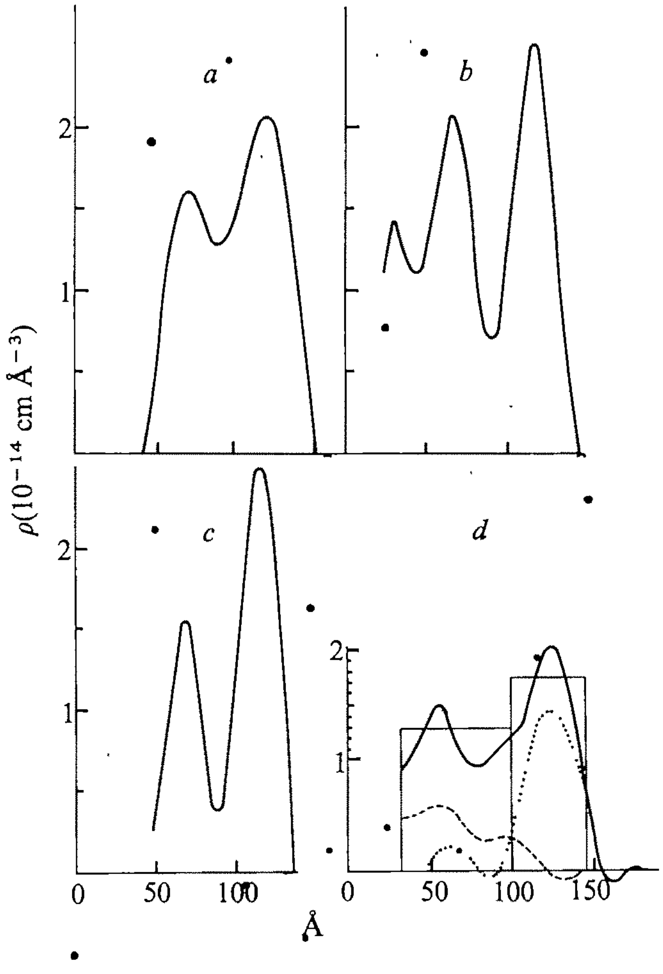
Another feature emerges from Table 2: with the exception of swollen BMV, at high *p*H, the first subsidiary maximum of the patterns dominated by the diffraction of RNA (in 43% D_2O) is at nearly the same positon for all of these viruses and, corresponds to an overall diameter of the RNA region of about 100 Å.

(3) The radial distribution of protein and RNA

The radial scattering density ρ(*r*) of the particles gives a more detailed picture. For an object with spherical symmetry,

$$\rho(r)-\rho_s=\frac{2}{r}\int_0^\infty sF(s)\,\sin 2\pi rs\,\mathrm{d}s$$

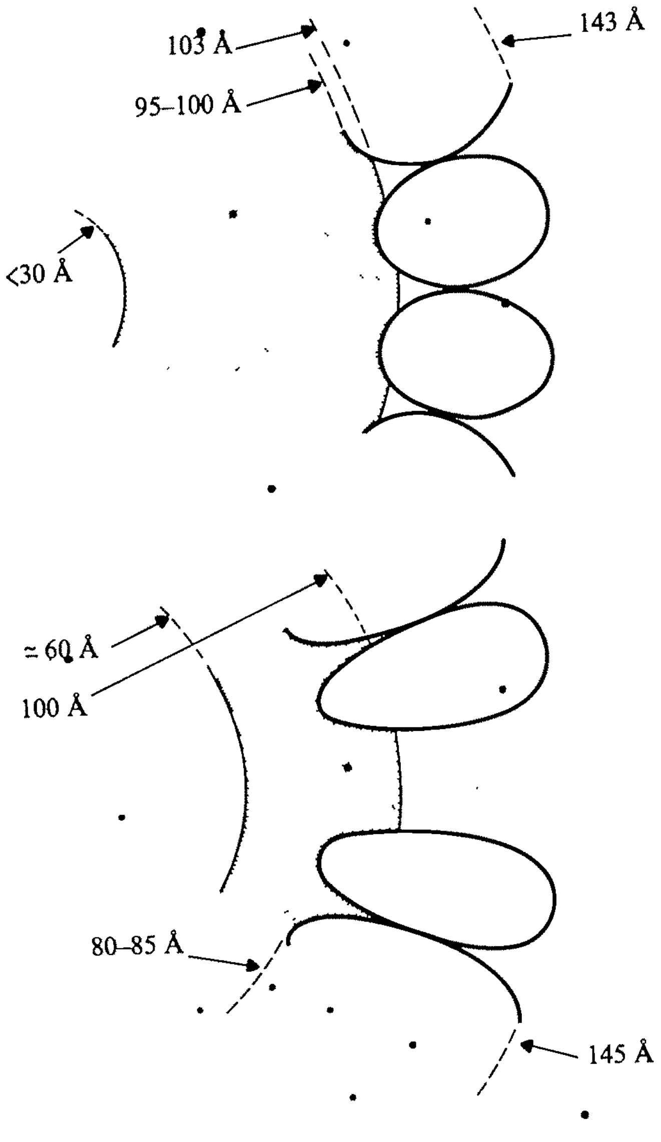
Fig. 2 Radial densities obtained by spherical Fourier transformation of structure factors deduced from scattered intensities: *a*, CMV in H_2O; *b*, TYMV in H_2O; *c*, MS_2 in H_2O; *d*, SBMV:——, in H_2O, – – – –, in 43% D_2O, ..., in 69% D_2O. The rectangles give the model used for the calculated curve (Fig. 1). All solvents were buffers of low molarity (0.02 M or below).



where ρ_s is the scattering density of the solvent. $F(s)$ is the square-root of $I(s)$, and its sign is assumed to change at each minimum of $I(s)$ as a consequence of the principle of minimum wavelength[19]. It is pertinent to compute $\rho(r)$ from data collected in H_2O and in buffers matching the contrasts of the protein and RNA moieties (that is, about 43 and 68% D_2O).

In Fig. 2 some of these radial densities are shown on an absolute scale. The variation with contrast of the protein part (the peaks at large radius), and of the RNA part (the more complex structure between the centre and the protein shell) confirms the attribution of the density to the corresponding moiety. These curves are deformed by cut-off effects in Fourier transforms and no reliable information can be obtained near the centre (r < 50 Å). The density at the maxima of the protein peaks, for instance in H_2O, is rather independent of the cut-off (Table 3). For TYMV and MS2 the experimental value of the density of this peak is that calculated for their coat protein, indicating a very tight packing of the subunits in the capsid. In contrast, for CMV and SBMV the experimental values are somewhat lower, suggesting the presence of channels between subunits in the capsid. The position and width of the protein peak is also very well determined, both by the radial density in H_2O and in 68% D_2O (Table 3). Except for SBMV they agree with those obtained from the analysis of the position and intensity of the first subsidiary maximum.

Fig. 3 Sketch of the organisation of protein and RNA in two viruses as deduced from neutron low-angle scattering data. The shaded area corresponds to RNA. *a*, TYMV. Protein molecules are represented in close contact. RNA does not penetrate deeply between subunits of the capsid *b*, CMV. The shape of the protein shell has been chosen to fit the data of Table 3. The size of the holes in the capsid has been calculated assuming 32 large holes (in the centres of hexamers and pentamers of the capsid). In this model the total volume accessible to RNA is about 2.5×10^6 Å^3; its average scattering density when this space is uniformly filled with CMV RNA is 1.60×10^{-14} cm Å^3, in excellent agreement with the experimental value.



For the RNA, which is located nearer the centre of the virus, the results are not so reliable. First, at contrast matching of the protein, the diffraction pattern has a fairly low intensity, and, except for SBMV, where a very concentrated sample was available, does not extend very far. Second, for a given resolution, Fourier transforms give less reliable results at smaller radii. The position of the RNA peak (Table 3) is correlated with the estimated size of a central hole as expected. The density of this RNA peak is also given in Table 3. Its accuracy is less than for the protein peak, but it is much lower than the density of pure RNA (4.04×10^{-6} Å^{-2}), indicating that 50–60% of the volume is occupied by water, even in the region where the RNA is the most closely packed.

Discussion

The analysis of the neutron diffraction at small angle by spherical viruses in solutions of buffer containing different amounts of D_2O provides a consistent set of data, from which low-resolution models can be built.

The comparison between values obtained for the dimensions and the density of the two moieties contributing to those viruses show the following features:

(1) All viruses studied in this work (with the possible exception of TYMV) possess a central hole, of increasing size in viruses containing relatively less RNA: Within 10% the difference between the overall RNA radius (Table 3) and the radius of the hole is proportional to the molecular weight of the RNA in the virion.

(2) The external diameter of the bulk of the RNA is much smaller than the virus diameter.

(3) The degree of interpenetration of RNA and protein is different in TYMV and CMV. In TYMV there is little (if any) penetration of the RNA into the protein capsid (about 5 Å), whereas in CMV this penetration must be about 20 Å. This difference in penetration is due to a difference in the internal diameters of the capsids. Our model for TYMV is different from that of Klug *et al.*[4], who suggested a strong imbedding of TYMV RNA into the capsid. The reason for this discrepancy will be discussed elsewhere[16]. We simply note here that our method gives in a fairly direct way the overall size of the RNA inside the virion, and that the high ionic strength used by Finch and Klug could induce a swelling of the RNA.

(4) We found that in TYMV (and MS2), the maximum of the scattering density of the protein shell suggests a very dense packing, whereas for CMV the packing is less dense, leaving room for holes representing about 15% of the surface at the radius of higher density (117 Å). The presence of such holes could explain the sensitivity of CMV to ribonuclease, even if its RNA is localised in a sphere much smaller than the overall size of the virus. These differences between TYMV and CMV organisation could account for the difference between their properties. The isomorphism of TYMV and TYMV empty capsids, the high stability of these capsids and virions, and the insensitivity of TYMV to ribonuclease, can be well understood from the structure we found for the virion (Fig. 4). Conversely, the absence of empty capsids, the sensitivity to ribonuclease and to magnesium ions can be easily understood from the model we propose here for CMV.

MS2 seems similar to TYMV, in agreement with the known properties of that virus, in particular the possibility of obtaining empty capsids by alkaline treatment. Our data for SBMV suggest that this virus may have some features in common with CMV, in particular, the presence of holes in its capsid: its sensitivity to ribonuclease is suggested by other experiments[21].

The case of BMV (ref. 25) is clearly intermediate between those of TYMV and CMV. In its low pH form it shows a small RNA penetration into the capsid (but larger than for TYMV or MS2). In its swollen, high pH form (which is likely to be an *in vitro* artefact), the RNA is strongly embedded between protein subunits. The fact that the BMV empty capsids, which can be formed at pH 4–5 (refs 22, 23), have a diameter intermediate between those of the two forms of the virion[24], suggests that both protein–protein and protein–RNA interactions contribute to the stability of that virus.

(5) If neutron low-angle scattering curves of virus solutions provide direct information on the protein–RNA interpenetration and on the density of the protein shell, very little can be said on the folding and the symmetry of the RNA at its contact with the protein shell. But it is clear that the RNA is, on average, loosely packed; the highest radial density measured corresponds to about 50% RNA, 50% solvent by volume.

In conclusion, neutron small-angle scattering of virus solutions allows a fairly clear correlation to be made for small RNA viruses between the packing of RNA and protein subunits, and the nature and importance of stabilisation forces. Deeper penetration of the RNA into the capsid seems to be correlated with the increasing importance of RNA–protein interactions. The technique, however cannot give data on the folding of the RNA. This could be achieved by neutron diffraction studies at low resolution of single crystals in various D_2O environments which will be undertaken soon.

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Collagen–mineral axial relationship in calcified turkey leg tendon by X-ray and neutron diffraction

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The axial relationship between the collagen and mineral components in calcified turkey leg tendon was investigated using low-angle X-ray and neutron diffraction. The results indicate that the mineral is arranged with the same axial periodicity as the collagen and occupies the gap region of the collagen structure.

MINERALISED connective tissue such as bone is a composite structure of protein, mineral and glycoproteins. The structural relationships between these components is still unclear although, individually, they are better understood.

In most hard tissues, the protein matrix is collagen which exists as long fibrils of diameter 200–1,000 Å and displays the 670 Å period of transverse bands seen in electron micrographs of normal tendon collagen[1,2].

The mineral is widely accepted as being calcium phosphate although there is disagreement as to the actual forms present. Crystalline hydroxyapatite seems to dominate[3] but there is clear evidence that an amorphous phase is also present[4]. X-ray[5] and electron microscope[6] studies show that the crystals have their unit cells oriented in a constant manner to the collagen fibril axis, suggesting a close structural relationship between these two ordered components. It is widely agreed that the crystals have one long side oriented parallel to the collagen fibrils, and the length of this long side is quoted variously between 300–1,000 Å. It is also agreed that one lateral dimension of the crystals is about 40 Å, although it is not known whether they exist as rods or plates. Many accept that the crystals are distributed along the collagen fibrils with the same 670 Å periodicity as collagen, but there is debate as to whether they are confined to the periphery or penetrate through the interior, and there is no certain knowledge about the axial location of the crystals with respect to the collagen period, although it has been suggested that they occur in the gap region[8].

We are investigating these questions using low-angle X-ray and neutron diffraction of mineralised turkey leg tendon. This tissue was chosen because the collagen is highly ordered in the axial direction, therefore giving well oriented diffraction patterns. Also, the system has been well studied using electron microscopy[8]. Although not strictly bone, the tissue does nevertheless represent a simple collagen–mineral interactive system, the results from which can be usefully applied to the whole problem of calcified tissues.

Principles of the method

X-ray and neutron diffraction patterns from tendons contain, at low angles, a set of meridional reflections which index as orders of 670 Å (D), the periodicity of the collagen fibrils when projected on to the fibril axis. The D period arises because, in projection, the 2,990 Å (L) long collagen molecules are staggered axially by D with respect to each other (Fig. 1). Since L is approximately $4.5D$, this molecular arrangement implies that each period has an overlap and gap region, each of approximately $0.5D$ in length.

The amino acid sequence of the collagen molecules is now known in some detail for rat and calf skin[10,11]. It is therefore possible to use the sequence and the Hodge–Petruska scheme

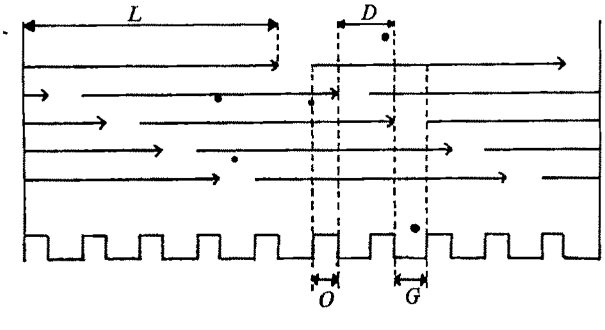

Fig. 1 Hodge–Petruska scheme for the molecular arrangement of collagen molecules[11]. *L* (2,990Å) is the length of each collagen molecule and adjacent molecules are staggered by *D* (670Å) with respect to each other. When projected on to the fibre axis, the arrangement produces a step function of period *D* containing an overlap region (O) and a gap region (*G*).

to construct the axially projected electron density (X-ray diffraction) and nuclear scattering density (neutron diffraction) profiles. From these models, the predicted intensities of the low angle X-ray and neutron meridional reflections may be calculated. It has been demonstrated[12,13] that a good correspondence can be obtained between the calculated and observed intensities in both diffraction techniques. Uncalcified turkey leg tendons from young birds show a meridional pattern very similar to that of rat tail tendon, the major differences occurring at a resolution in the order of 20 Å. This represents a much higher resolution than will be described here (74 Å) and, therefore, the scattering density models obtained for the rat tail can be confidently applied to this study of the turkey tendon. Thus, by comparing the X-ray and neutron diffraction patterns of calcified and uncalcified tendon an attempt can be made to locate the mineral with respect to the collagen axial structure.

The major problem when trying to compare the diffracted intensities from two similarly periodic structures containing different amounts of scattering material is one of scaling. In this case, it was overcome (in both diffraction techniques) by measuring the relative first-order intensities of a sample before and after decalcification. Allowance was made for the different absorption properties of the specimen with and without the mineral by normalising the intensities to the measured transmission of the undeviated beam. The important assumption that decalcified and uncalcified turkey tendon collagen are equivalent is substantiated by their X-ray diffraction patterns, which show no measureable differences to a resolution of 15 Å. Decalcification was carried out in phosphate-buffered EDTA (*p*H 8.0; 5%) followed by extensive washing in 0.15 M NaCl.

All experiments were carried out on leg tendons freshly dissected from turkeys killed in week 22 after birth. The tendons begin to calcify at week 16 and in the following 6 weeks calcify extensively to form stiff bone-like structures.

X-ray diffraction

Previous low-angle X-ray work[14] on calcified systems, including the turkey leg tendon, has shown the existence of an axial repeat of spacing 660 Å, and the intensities of the first six orders have been quantified. It was observed that these reflections have a much higher absolute intensity than those from uncalcified tendon. Furthermore, the reflections were obtained with mineralised tendon from which the collagen had been removed. It was concluded that these reflections come from the mineral component distributed with the *D* period and not the collagen which exhibits a spacing of 640 Å in the dry state.

We have extended these observations. Figure 2 shows the low-angle meridional patterns from calcified and uncalcified turkey leg tendon as well as that from rat tail tendon for comparison. Table 1 lists the measured intensities of the nine orders which are seen in the diffraction patterns. Several important preliminary observations can be made.

As observed by other workers[14], there is strong diffuse scatter from the mineralised tendon at low angles, which is confined to the equator. It is widely agreed that the origin of this scatter is the 'form factor' (or 'shape factor') of individual crystallites of mineral from which estimates of the crystal size can be made[7].

The absolute intensities of the reflections are indeed much higher for the tendon in the calcified state which must be due to the extra scattering by the mineral, although orders 1–5 in each pattern have relative intensities which are very similar.

Finally, the spacings of the reflections from the calcified and uncalcified tissues are exactly the same (orders of 670 Å). Because the mineral absorbs the X rays to such a large extent (approximately 90% attenuation for the thickness of sample used, see Fig. 2) and scatters much more strongly than collagen, it could be argued that the calcified tissue reflections are due solely to the mineral arranged *D* periodically, and that the collagen reflections which may exhibit a different *D* periodicity

Fig. 2 Low-angle X-ray diffraction patterns showing the first nine meridional reflections of rat tail tendon collagen (*a*), uncalcified turkey tendon collagen (*b*), calcified turkey tendon collagen (*c*). A mirror-monochromator focusing system was used linked to a rotating anode source (Elliot Automation GX6) with a specimen-to-film distance of 85 cm. Thin specimens, approximately 0.2 mm in diameter were suspended over 0.15 M NaCl in a sealed Perspex cell with Mylar windows. The rat tail tendon and the uncalcified turkey tendon clearly give very similar diffraction patterns. *d*, Calcified turkey tendon, taken at the Synchrotron Radiation Facility (EMBL) at DESY, Hamburg, in collaboration with A. Harmsen (specimen-to-film distance was 39 cm). The higher orders of the collagen are just visible above the intense low-angle diffuse scatter from the mineral crystallites.

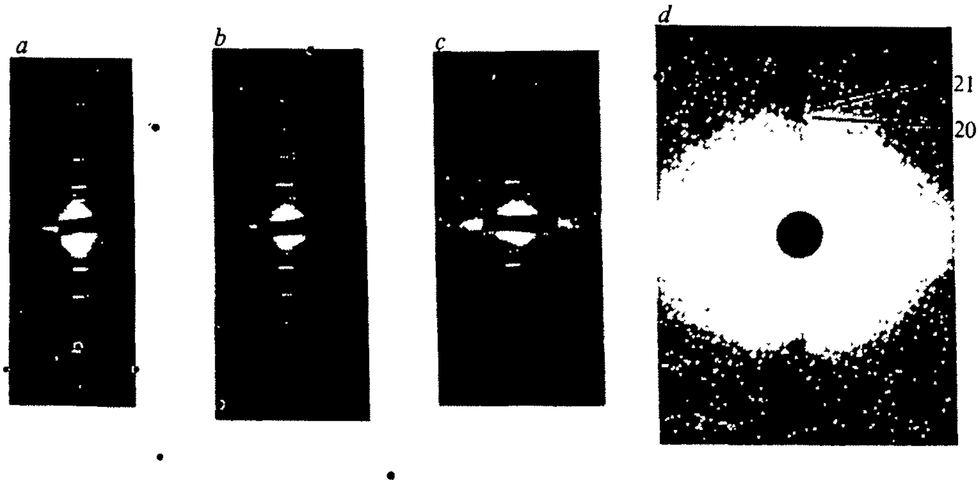

Table 1 Intensities of the first nine orders of the meridional X-ray diffraction patterns from rat tail tendon, uncalcified turkey tendon and calcified turkey tendon

Order	Rat tail tendon	Turkey tendon uncalcified	Turkey tendon calcified	Calcified collagen model
1	1,000	1,000	9,145	9,145
2	27	9	102	97
3	109	107	834	993
4	11	7	68	72
5	54	48	192	365
6	17	8	81	88
7	21	30	51	140
8	6	8	17	35
9	71	37	59	55

To measure intensities accurately, each diffraction pattern was digitised on an Optronics densitometer system, and the numerical data analysed with a computer program which integrates over each reflection and subtracts local background. Various corrections were applied to allow for the geometry of the X-ray camera, and other factors inherent in the measurement of fibre diffraction patterns. These are fully described elsewhere[15]. The arbitrary scale is 1,000 for the first order of uncalcified collagen. The final column lists the calculated intensities as obtained from the refined model of the calcified collagen.

are too weak to be seen. Very long exposures, however, fail to show up any other low-angle reflections. Furthermore. Fig. 2 also shows a diffraction pattern of mineralised turkey leg tendon obtained with Synchrotron Radiation at DESY in Hamburg. This very high-intensity X-ray source allows large specimen–film distances which reduces the diffuse scatter relative to the Bragg peaks whilst still retaining the ability to view the weak collagen reflections at higher angles. The higher orders of collagen, although still very weak, are clearly visible on the 670-Å spacing, especially the characteristically strong 20th and 21st.

Several important conclusions can be made. First, the mineral is arranged *D* periodically in the form of a step function along the collagen. The step width must be about 0.5*D* to give the strong, first, third and fifth orders. Second, the underlying collagen matrix retains its wet, native periodicity of 670 Å. At higher resolution, where the transform of the mineral component step quickly dies away, the collagen pattern can be seen.

The important question concerning the relationship between the two components, however, still remains unresolved. A specific interaction would mean that the amplitudes of the two individual transforms would add, whereas an incoherent arrangement would cause the intensities to add. The distinction cannot be readily made because the mineral scatter is so dominant. To help overcome this problem, the material was studied with neutrons.

Neutron diffraction

This is a particularly attractive technique to apply to calcified systems for two reasons. Unlike X rays, the nuclear scattering of neutrons by mineral and protein is comparable, and the former component does not dominate the resultant pattern. Second, we can use the fact that the background scattering by the water can be altered by introducing various amounts of D_2O into the system—the so-called contrast variation technique[16]. The average scattering length density of collagen at low resolution equals that of an aqueous background containing 47% D_2O, whereas that of the mineral is shown below to occur at a much higher value in the region of 80%. This large separation of 'matchpoints' between the two components means that we can effectively look at the scattering from one independently of the other. The behaviour of the rat tail collagen neutron diffraction pattern at different H_2O/D_2O ratios is well understood[17] and can be used to interpret the results from the calcified collagenous system.

The first six orders from calcified turkey tendon were collected on the D-11 instrument[18] at the Institut Laue-Langevin, Grenoble in a range of D_2O–H_2O mixtures from 0 to 100% in steps of 20% (Table 2). Several important features of the diffraction pattern and its variation with percentage D_2O are clear.

At all contrasts, except that in 100% H_2O, the intensities indicate that the structure in projection has a step function nature in having relatively strong first and third orders and a weaker second. Nowhere, however, is it as pronounced as with the X-ray data, and this reflects the fact that with neutrons the mineral is not dominating the diffraction pattern.

If the intensities are converted to amplitudes (by taking the square root), it is possible to plot their variation with percentage D_2O for each order and in all cases the relationship is non-linear. For any centrosymmetric structure, it can be shown that the amplitudes of its Fourier transform will vary linearly with percentage D_2O (ref. 16) and this is true for the low resolution rat tail tendon data (unpublished results). Clearly, the calcified collagen structure in projection is not centrosymmetric.

The large increase in the sixth order is entirely consistent with the collagen component and essentially results from its amino acid sequence[17].

Finally, the first-order intensity of calcified collagen is actually less than that of uncalcified collagen at 0% D_2O. If the intensities from each component were merely adding to produce the composite value, the first order for the calcified should be much higher than that of the uncalcified. The fact that this is not the case, strongly suggests that the amplitudes are adding with some phase relationship which in turn implies a specific interaction between the collagen and the mineral.

The results can be further analysed to give a low resolution picture of the relationship between the collagen and the mineral.

The X-ray results indicate that, at the resolution of the first order, both components can be considered as step functions. The first order of the composite diffraction pattern is a resultant of the two step function transforms at some phase angle Q. Therefore, determination of Q will give the axial relationship between each component. Contrast variation makes this possible using the neutron results.

Let A_M and A_C be the mineral and collagen first-order amplitudes in 100% H_2O, F_M and F_C the fractions of D_2O at which each amplitude falls to zero (the respective matchpoints), and A_{XM} and A_{XC} the amplitude values at a fraction X of D_2O. Each component, being a step function, is centrosymmetric and the amplitudes of their transforms will vary linearly with the D_2O composition. It follows that

$$A_{XM} = A_M - (A_M/F_M)\,X \tag{1}$$

$$A_{XC} = A_C - (A_C/F_C)\,X \tag{2}$$

Table 2 Variation of the first six orders of calcified turkey tendon meridional neutron diffraction pattern with percentage D_2O in the medium

Order	Calcified turkey tendon intensities					
	% D_2O					
	0	20	40	60	80	100
1	861	481	349	357	659	979
2	25	13	10	15	30	42
3	29	29	17	30	84	139
4	4	3	5	11	20	26
5	10	8	2	4	13	21
6	4	6	13	31	61	85

Specimens of calcified tendon were prepared by laying slices of tendon side by side to form a sheet approximately 0.1 cm thick and 2 cm square. Large specimens are required to use fully the 1-cm² neutron beam. After thorough equilibriation in the 0.15 M NaCl DO_2/H_2O mixture, the sheets were mounted in specially prepared sealed cells with quartz windows containing the same bathing medium. Six orders could be well resolved on the 64 × 64 cm² detector using a wavelength of 5.4 Å, and a specimen–detector distance of 5.53 m. As with the X-ray data, the numerical output from the detector was analysed by a program which calculates integrated intensities corrected for local background. All values are scaled relative to a first order value of 1,000 for rat tail tendon in 100% H_2O.

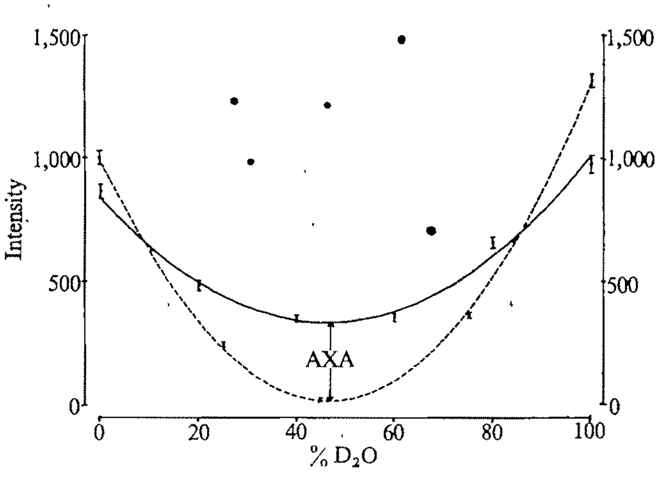

Fig. 3 Variation in first-order intensities of calcified turkey tendon (continuous line) and rat tail tendon (broken line) with percentage D_2O. The quadratic curves were fitted to the observed data using a least squares routine. The first cross-over point at 8% D_2O represents a point where the length of the two component amplitudes is such that their resultant is equal in length to the collagen amplitude alone. The second cross-over point at 86% D_2O represents the matchpoint of the mineral phase. At 47% D_2O where the collagen contribution is almost zero, one can estimate A_{XM} the mineral amplitude alone at this particular percentage D_2O.

Using the cosine rule, the resultant intensity variation of the composite first order with D_2O is

$$\begin{aligned}A^2{}_{MCX} = {} & X^2((A^2{}_C/F^2{}_C)+(A^2{}_M/F^2{}_M)+(2A_CA_M/F_CF_M)\cos Q)\\ & -X((2A^2{}_C/F_C)+(2A^2{}_M/F_M)+(2A_CA_M/F_M)\cos Q+\\ & +(2A_CA_M/F_C)\cos Q)+(A^2{}_C+A^2{}_M+2A_CA_M\cos Q)\end{aligned} \quad (3)$$

This equation predicts a quadratic variation in the first-order intensity with respect to fractional D_2O, and Fig. 3 shows a quadratic curve fitted to the results together with the rat tail tendon first-order intensity variation. The cross-over point provides a value for the mineral matchpoint F_M (0.86 D_2O). At 0.47 D_2O, the intensity of calcified system gives an estimate of A_{XM} (18.3) where the collagen contribution is at a minimum, and therefore A_M can be calculated from equation (1) as 40.4. A_C and F_C (31.42 and 0.47 D_2O) are known from the rat tail tendon data (unpublished results). Using equation (3), a value for Q can be found which gives maximum agreement with the quadratic curve fitted to the data shown in Fig. 3. The value resulting from such an analysis is 134°.

Refinement

Can this very crude model of two-step functions related by a phase angle of 134° be improved? The nine orders of the X-ray pattern of calcified tendon provides higher resolution information than the six orders observed using neutrons, and these can be used to refine the structure. Although the first few orders are dominated by the mineral, this is not true of the higher orders where the mineral transform is quickly dying away. The model structure for rat tail tendon collagen provides a reliable source of phases of the first nine orders of the X-ray pattern which can be combined with observed intensities to give a low resolution model of the uncalcified tendon. By adding a step function to represent the mineral, it is possible to calculate the expected X-ray intensities from the composite system and compare them with the observed. The length of the step function was varied between 0.45*D* and 0.55*D* (as indicated by the X-ray results), and its position varied between 120°→170° and −120°→−170° relative to the collagen step function (as indicated by the neutron results). Both angular regions need to be included because the centrosymmetric assumption used in the neutron diffraction data analysis cannot distinguish between these two possibilities. For convenience, observed and calculated first-order intensities were equalised by adjusting the height of the mineral block, and an R factor $\Sigma(|F_{obs}|-|F_{calc}|)/\Sigma|F_{obs}|$ was calculated on the amplitudes of the other eight orders to provide a measure of agreement.

The average R factor obtained within the refinement region was 0.49, but with a step width of 0.48*D* and a phase shift of +160°, a minimum value of 0.17 was obtained. This minimum is 2.8 s.d. units from the mean. For structures chosen randomly outside the refinement region nowhere was a value less than 0.6 observed. The calculated intensities from the best fit model are listed in Table 1 for comparison with the observed data.

Figure 4 is a Fourier synthesis of the composite structure using the model phases and observed X-ray amplitudes.

Discussion

The axially projected structure proposed for the calcified turkey tendon shown in Fig. 4 is based primarily on the results of X-ray diffraction, but it is consistent with the neutron diffraction. The observation that the first-order intensity with neutrons in 100% H_2O is higher for the uncalcified than for the calcified tendon is consistent with the component step functions being 160° out of phase, and the neutron scattering from each being comparable. In effect, the mineral scatter is cancelling that of the collagen and, therefore, removal of the former increases the first-order intensity. This also explains why the step function is enhanced at higher percentages of D_2O in the medium. In the middle range near the collagen matchpoint (30–60%) where its contribution is small, the mineral step dominates; the reverse is true in the higher range (70–100%) near the mineral matchpoint.

As predicted, the structure is not centrosymmetric, which would only be the case if the phase angle between the two first order components were close to 0° or 180°. The disparity between the phase angles obtained from the two diffraction techniques may seem rather large. The difference of 26° only represents, however, an axial distance of 48 Å which is less than the nine-order (74 Å) resolution of the model, and the value of 134° obtained from the neutron data analysis did provide a useful starting point for the refinement using the higher resolution X-ray data.

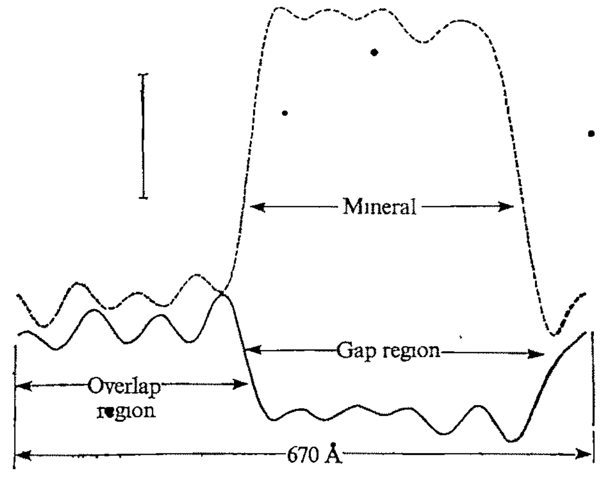

Fig. 4 Fourier syntheses of the uncalcified (continuous line) and calcified (broken line) turkey tendon with a *D* period Both were constructed using the observed intensities and calculated phases of the first nine orders—the uncalcified collagen phases from the rat tail tendon model, and the calcified collagen phases from the refined model described in the text. For clarity, the two curves are slightly offset, but the overlap region of the uncalcified collagen corresponds in electron density to the region of the calcified profile where there is no mineral contribution. Exact axial register between the minor peaks on each curve is not to be expected at this poor resolution where distortion due to termination errors is very high. It is clear, however, that the mineral is lying in precise axial alignment with the gap region. The vertical bar represents a height of 50 electrons per residue translation.

This work has shown that a definite axial relationship exists between mineral and collagen in calcified turkey leg tendon. The most attractive feature of the model is that the size of the mineral block and its position places it precisely in the gap region. Although these results provide no information concerning the lateral arrangement of the two components, it is difficult not to speculate that the mineral does penetrate the fibril and occupy the gap region. As indicated by others[8], this is the only way that penetration of the collagen by the mineral could occur without disrupting its fibrillar structure. The height and length of the artificial step, used to represent the inorganic phase in the model, corresponds to an actual volume of about 30,000 Å^3 per collagen molecule (using the apatite electron density). Taking the mineral block length as 0.48D (320 Å), this would correspond to a cross-sectional area of 94 Å^2. This may be considered rather low when compared with the crystal size estimates mentioned earlier, but it must be stressed that the tendons from 22-week-old birds are certainly not fully calcified, and that this value of 30,000 Å^3 only represents some kind of average over the tendon. A quantitative chemical analysis of the amount of mineral present in the tendons of birds at various ages should help to clarify this point.

Preliminary X-ray pictures of bat phalangeal bone indicate a similar low-angle diffraction pattern to that of calcified turkey tendon, and it is hoped that work on this and similarly well oriented hard tissues will bridge the gap between the calcified tendon system and true bone.

The initial X-ray work described was carried out in the Laboratory of Molecular Biophysics, University of Oxford, and we thank Professor D. C. Phillips, Dr C. Rodger and Dr B. Brodsky (Doyle) for their advice and assistance during this period. We thank the Arthritis and Rheumatism Council and the European Molecular Biology Organisation for support, and the ILL for neutron scattering and computing facilities.

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letters to nature

The possible galactic origin of γ-ray bursts

FOLLOWING the discovery of non-solar γ-ray bursts[1], various models to account for their origin have been discussed. Extragalactic models include supernova bursts in remote galaxies[2], and the collapse of the cores in active stars[3], while galactic models are based on flare stars[4], thermonuclear explosions on neutron stars[5] and the sudden accretion of cometary gas on to neutron stars[6]. The acceptability of any of these models may be tested by the observed size spectrum of γ-ray bursts. The extragalactic models predict a power law spectrum with $\alpha = -1.5$ while for the galactic models the number index will be -1. The experimental data on γ-ray bursts is still meagre. So far, about 44 confirmed events have been recorded by satellite-borne instruments[7,8]. The number spectrum of the observed γ-ray bursts (Fig. 1) shows that the observed distribution for events with an energy less than 10^{-4} erg cm^{-2} is flat and thus makes the choice of any model completely arbitrary Carter *et al.*[9] presented an upper limit on the number of γ-ray bursts having an energy $> 2.10^{-7}$ erg cm^{-2} and suggested that the data are consistent with a power law spectrum in which $\alpha \sim 0.5$, or a modified galactic distribution. We present here an alternative analysis of the observed γ-ray events, which suggests very interesting possibilities for their origin.

In Table 1 we have listed the chronological catalogue of the observed γ-ray events. Inspection of the data relating to the estimated flux suggests that there is a preferred mean energy for γ-ray bursts. Some 90% of the events have an energy between 5×10^{-5} and 5×10^{-4} erg cm^{-2}, contrary to the predicted characteristics of the number spectrum of various models even if a detection bias of 10 is taken into account for low energy bursts.

We estimate in this analysis the relative distance of the origin of each burst by assuming a constant intrinsic luminosity of the source. The data were then divided into 10 bins. The number of bins is chosen to reflect the observed variations in energy by two orders of magnitude. In the histogram obtained from this analysis (Fig. 2*a*), the numbers are plotted using an arbitrary distance scale. In Fig. 2*b* we have plotted the galactic distribution of HII regions and clouds of molecular hydrogen[11]. The remarkable similarity between the distribution of γ-ray bursts, and that of supernova remnants, suggests a genetic relationship between the two and the galactic origin of the γ-ray bursts. A selection bias in binning the data to generate the γ-ray burst histogram can alter the shape slightly but the overall gaussian nature is preserved under all combinations.

If the apparent correlation shown in Fig 2*a* between γ-ray bursts and supernova remnants is genuine, then the burst source could be identified with 'cool', that is, completely run down neutron stars. Current theories of supernovae suggest that each supernova explosion results in the formation of a neutron star. This prediction has been further supported by the discovery of pulsars About 20% of these compact objects will be in close binary systems. Most of these neutron stars will lose their angular momentum in 10^4–10^6 yr, because $\Omega/\Omega = P/P = 10^{-12}$ s, is representative of the observed values of P in various pulsars. Thus, at any time, there exists a large population of 'cool' neutron stars in our Galaxy. We propose that γ-ray bursts originate from the disruption of these 'cool' neutron stars.

Lattimar and Schramm[12] investigated a mechanism whereby a neutron star falling towards a black hole is tidally disrupted, for example, in a binary system. This results in the decompression of the ejected neutron-star matter. As this material expands it follows a unique evolutionary path, like the r-process,

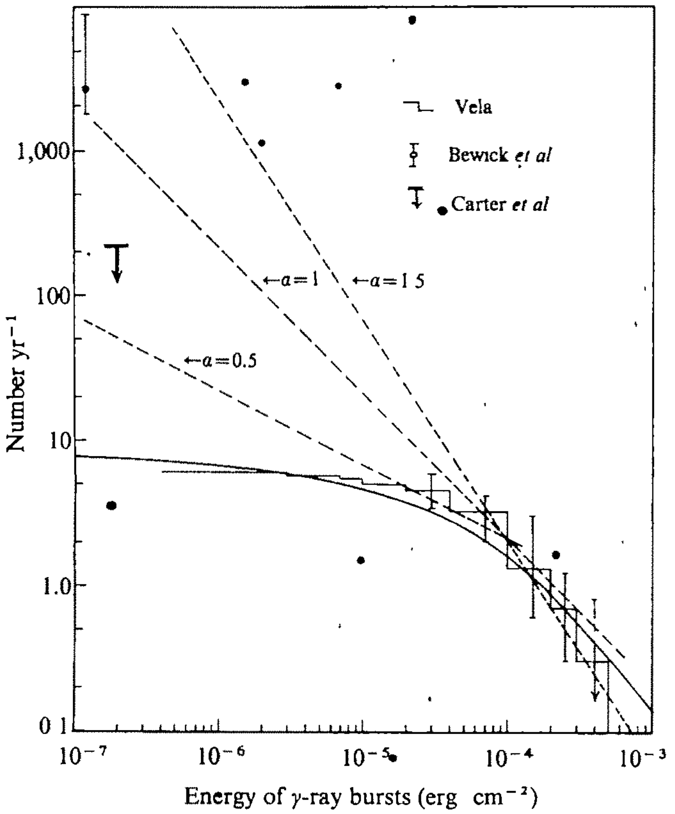

Fig. 1 The number spectrum of the observed γ-ray bursts. The three dotted lines labelled $\alpha = 1.5$, 1 and 0.5 represent the power law function $N \propto S^{-\alpha}$, predicted for sources distributed uniformly in 3, 2 and 1 dimensions, respectively. ——, Cumulative gaussian distribution normalised at 5×10^{-4} erg cm^{-2}.

towards the region of superheavy elements[13], and nuclei up to mass $A \sim 300$ are formed. Fission then sets in until such time that all the neutrons are used up. This process would result in a γ flash. The characteristic time scale for such a process is from 10 ms to a few seconds. If we take account of the comparatively long decay times of the various isotopes and the β-decay life time of the various intermediate nuclei, the observed time structure of γ-ray bursts can easily be accounted for. It is interesting, however, that some of the observed features will follow naturally from such a model.

(1) The spectral shape for all events will be similar. This is suggested by the spectra of the 14 events observed by the IMP satellite[8].
(2) The energy released during each disruption will be similar, as there is only a small range in the permitted mass of neutron stars.
(3) The expected rate of occurrence of γ-ray bursts in the entire energy range should remain constant within statistical limits.

The present model can also provide the required energy, which at a distance of 6 kpc and an average observed energy of 5×10^{-4} erg cm^{-2}, corresponds to 10^{42} erg s^{-1} emitted in γ rays during the burst. Assuming that 1% of the total energy is radiated as γ rays, the total energy released corresponds to 10^{44} erg s^{-1}. This value is well within the range of the energies associated with stellar explosions. As the energy is released in the disruption of the core, the restrictions observed in accreting models due to the Eddington limit ($\sim 10^{38}$ erg for the case of a neutron star having a mass of $1 M_\odot$) do not apply.

It is possible to predict the form of a number spectrum of the observed events if the association with supernova remnants is valid and neutron star disruption results in γ-ray bursts. The number spectrum predicted by such a model can be defined as a product of three distributions.

$$N(E) = N_C(t) N_L(E) N_R(r)$$

where N_C is the number distribution for star disruption, N_L the distribution of the luminosities of burst sources, and N_R the distribution of sources with distance.

All three distributions in the equation can be approximated by gaussian functions. The first two represent statistical phenomena, and the observed distribution of N_R seems to be gaussian as is indicated in Fig. 2*a* Therefore, the observed composite distribution will also be gaussian. The integral number-spectrum of burst events per yr will then be represented by a cumulative gaussian function Figure 1 shows that such a distribution fits the observed number distribution extremely well.

The present model predicts an anisotropy in the arrival directions of the bursts. The magnitude of this anisotropy will depend on the spatial distribution of the 'cool' neutron stars. Any analysis of this nature is limited by the very small number of observed burst (50) and the fact that the arrival directions

Table 1 Chronological catalogue of γ-ray events

Serial No.	Date	Time (UT)	Estimated flux (Xfu)* Vela†	Imp‡
67–1	2 July 1967	14 19 28	100	
69–1	3 July 1969	07 17 13	20	
69–2	19 July 1969	03 46 46	100	
69–3	7 October 1969	07 26 31	200	
69–4	17 October 1969	03 18 47	20	
69–5	17 October 1969	21 41 53	40	
70–1	25 January 1969	05 01 30	—	
70–2	10 July 1970	05 17 46	40	
70–3	22 August 1970	16 49 31	100	
70–4	1 October 1970	15 42 16	100	
70–5	1 December 1970	20 00 59	4	
70–6	30 December 1970	07 02 17	300	
71–1	2 January 1971	19 10 56	100	
71–2	27 February 1971	17 27 35	70	
71–3	15 March 1971	11 20 27	50	
71–4	18 March 1971	15 28 05	100	
71–5	21 April 1971	03 18 39	3	
71–6	30 June 1971	17 30 59	500	
72–1	17 January 1972	17 39 16	70	
72–2	12 March 1972	15 53 15	50	
72–3	28 March 1972	13 46 28	100	
72–4	27 April 1972	10 58 32	30	
72–5	14 May 1972	03 46 31	200	
72–6	1 November 1972	18 56 46	7	
72–7	13 November 1972	16 02 38	10	41
72–8	18 December 1972	20 27 39	100	120
73–1	25 January 1973	15 14 57	3	46
73–2	2 March 1972	23 27 58	300	280
73–3	16 April 1973	12 38 57	30	30
73–4	7 May 1973	08 04 32	6	—
73–5	17 May 1973	01 34 38	—	36
73–6	6 June 1973	07 07 28	100	60
73–7	6 June 1973	18 47 14	—	71
73–8	10 June 1973	20 50 42	100	90
73–9	21 July 1973	08 55 18	200	120
73–10	25 July 1973	17 07 57	200	65
73–11	20 August 1973	16 39 16	—	27
73–12	18 September 1973	05 11 00	200	—
73–13	26 September 1973	16 04 31	30	70
73–14	17 December 1973	08 09 08	80	110
73–15	23 December 1973	00 39 47	80	62
74–1	21 January 1974	18 05 22	—	42
74–2	2 April 1974	18 20 38	—	41
74–3	19 April 1975§	17 14 35	—	30
74–4	28 July 1974§	14 01 46	—	27
74–5	6 August 1974§	10 36 37	—	420
74–6	23 August 1974	14 35 44	—	20
74–7	29 September 1974	18 51 45	300	—
74–8	14 December 1974§	10 14 53	—	—

*1 Xfu (X-ray flux unit) = 10^{-6} erg cm^{-2}.
†Energy range 150–750 keV (Vela 5) and 300–1,500 keV (Vela 6).
‡Measurements between 100 and 1,000 keV.
§Unconfirmed.

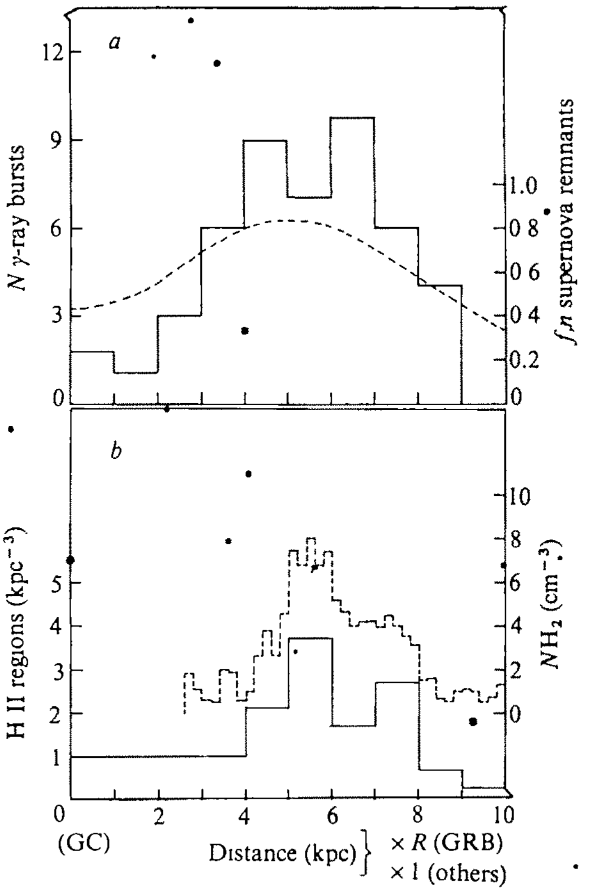

Fig. 2 *a*, The distance distribution of γ-ray bursts (——, arbitary scale with galactic centre (GC) at 10 kpc) is plotted along with the inferred galactic distribution of supernova remnants (- - - - - -)[10]. *b*, Radial distribution of other galactic constituents, H II regions (———) and the molecular hydrogen (— — —).

have large error boxes, except for a few cases. As most of the events were seen by the Vela satellites, the inferred positions have relatively larger errors in galactic longitude than in galactic latitude. Strong *et al.*[14] have analysed the latitude dependence of 16 γ-ray bursts. The resulting distribution deviates significantly from that expected on the basis of isotropic distribution, suggesting that γ-ray burst sources indeed cluster near the galactic equator. This conclusion is based on a small sample of observations, and therefore we do not yet regard this as firm supporting evidence.

The present apparent isotropic nature of γ-ray burst sources may be misleading due to poor statistics. More directional data are required to establish the form of the frequency distribution.

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Possible detection of a radio event correlated with a γ-ray burst

COSMIC bursts of electromagnetic radiation, both isolated and in connection with other impulsive astrophysical phenomena, have been sought for many years[1], with only one suggestive positive result[2], until, in 1973, the discovery of γ-ray bursts[3] of cosmic origin prompted a new series of observations[4]. In 1975 we started a systematic search for electromagnetic bursts at v.h.f. and u.h.f. The system, based at Medicina (Bologna, Italy), is semi-automatic and assures highly efficient data collection. Simultaneous observations at different frequencies facilitate the evaluation of disturbances of local origin and, hopefully, their isolation. Regular observations started in July 1976, and we report here our detection of a radio burst, which seems to be correlated with a γ-ray burst.

On August 16 1976, a radio burst was detected at Medicina at four different frequencies[5]. About 60 s earlier, three satellites (1 Vela, 2 Solrads) (R. W. Klebesadel, personal communication) and one balloon-borne detector on its second transatlantic flight (M. Sommer, personal communication) detected an intense γ-ray burst.

Details of the radio and γ data are given in Table 1.

The 151- and 408-MHz signals were detected by north–south arrays of 10 dipoles (HPBW = 15°×75° and 12°×75°, respectively), used as transit instruments pointing along the meridian at an altitude of 16.5°. The 323.5- and 330.5-MHz data were obtained using two receivers connected to a 7° HPBW parabola (10-m diameter) tracking the Sun. The parabola operated in connection with the X-ray detector on its second transatlantic balloon flight. From the recordings (Fig. 1) it seems that the pulses have a duration of 30 s, which is long compared with the response recovering time of the pen recorders. On the same charts are several interference spikes whose width, much narrower than this, results from the finite thickness of the pen trace.

The similar duration and coarse structure of the radio pulses, evident at all frequencies, the coincidence within the resolution time between the signals detected by the arrays and the Medicina parabola (which are 700 m apart from each other), and the detection at four different frequencies make a man-made origin of the signal very improbable.

Strong evidence of a non-local origin of the phenomenon comes from the independent observation carried out at the Osservatorio Astronomico of Trieste (400 km away) (Table 1). At 237 MHz, with a 15° HPBW parabola (10-m diameter) monitoring the Sun, a structured event above the noise appears on the record in coincidence with our radio burst.

An atmospheric origin seems unlikely, first because the weather was clear and fine when the burst was detected, and second, because of the time coincident event at Trieste. From the rate of isolated pulses seen in coincidence within 10 s by the parabola and the arrays at Medicina, we expect a rate of fourfold random coincidence of 1 per 24 h. If we consider the Trieste dish, the rate of fivefold random coincidences drops to 1 per 432 h.

The γ-ray event occurs ~60 s earlier. Assuming a coincidence time of 2×60 s, the probability of a random correlation between the γ burst and the fivefold radio coincidence is 8×10^{-6}. This supports the hypothesis that the radio signals are the counterpart of the γ event, although a definite correlation can be given only when the location of the γ and radio event is found. To

Table 1 Radio and γ data for August 16 1976

Detector type	Array of dipoles		Medicina parabola		Trieste parabola	Balloon X-ray telescope
Frequency (MHz)	151	408	323.5	330.5	237	—
Bandwidth (MHz)	1	2	2	2	2	—
Energy Range (keV)	—	—	—	—	—	50—250
Onset time (UT in h, min, s)	16.16.24 (±5 s)	16 16.24 (±5 s)	16.16 25 (±3 s)	16.16 25 (±3 s)	16.16.20 (±20 s)	16 15.27 —
Duration (s)	30	30	30	30	40	30
Integration time (s)	0.3	0.3	1	1	0.5	—
Peak intensity (W m^{-2} Hz^{-1})	8×10^{-22}	9×10^{-22}	3×10^{-23}	1.4×10^{-23}	2×10^{-22}	—

pinpoint the position of the radio burst source in the sky we could in principle convolve the heights of the signals measured at the various frequencies with the aerial beam patterns.

When the event occurred, the axes of the parabolae and of the arrays were roughly orthogonal. The relative peak intensities of the pulses cannot be reconciled with the source on the axis of any of the aerials. The Sun is then excluded, being on the axis of the two parabolae. A non-solar origin of the radio event is also supported by the available information on the Sun: on August 16 there was no evidence of activity in H (ref. 6), while at radio frequencies only a weak solar storm, not polarised, was present. Furthermore, a purely phenomenological analysis of the signals seen at the five different frequencies excludes the hypothesis of a typical solar radio burst.

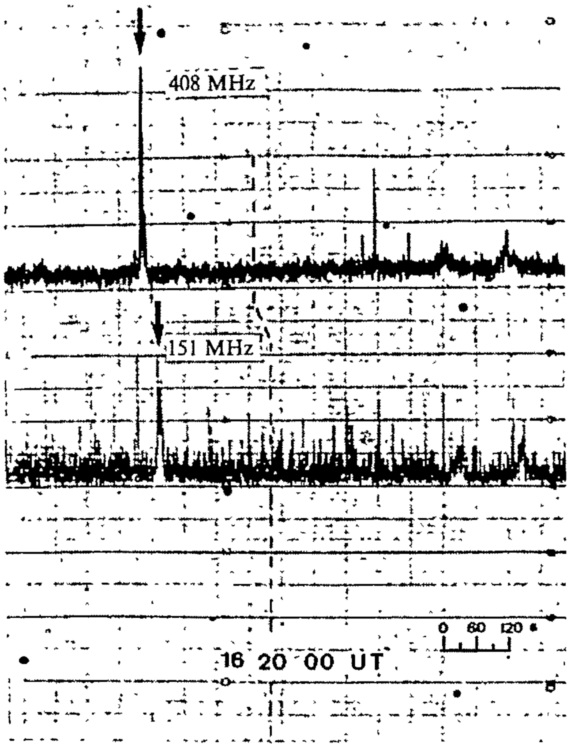

Fig. 1 Signals recorded on August 16 at 151 and 408 MHz. (Chart speed 60 cm h^{-1}.)

The decreasing peak intensities of the signal detected at 237, 323.5 and 330.5 MHz, respectively, and the frequency dependence of the parabola beam shape suggest that the source was at least 8° off the Sun position.

Analysis of the general trend of the power patterns of the aerials and their relative positions shows that the beam overlaps were only marginal. Here, the influence of side lobes is dominant and difficult to measure. Precise calculations of the beam pattern intersections are therefore useless and probably misleading Hence we limit ourselves to defining a region of sky (shaded area in Fig. 2) where the radio burst most probably originated. Some interesting objects exist in this region. Its area is so large, however, that any attempt to identify a precise object is meaningless.

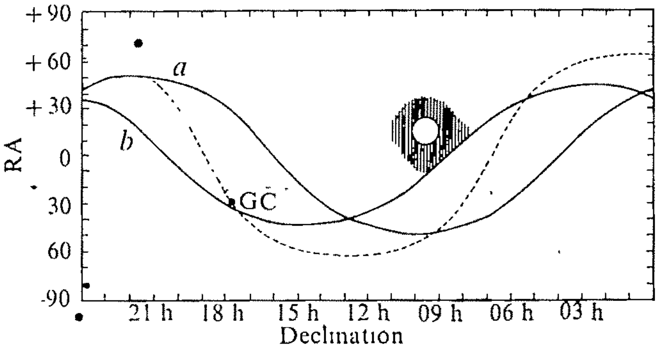

Fig. 2 *a*, Medicina horizon; *b*, balloon horizons, in equatorial coordinates The radio burst location is marked by the shaded area. — — —, Galactic equator, GC, Galactic centre.

We notice that all the possible positions of the source are at least 15° above the galactic plane and a delay between the 151- and 408-MHz radio signals produced by interstellar dispersion not greater than 10 s must be expected. It agrees with the upper limit we can assign to the delay between the extreme frequencies used in our experiment. The 1-min time difference between the onsets of γ and radio signals cannot be produced by dispersion. It implies a much higher delay between the radio signals. The γ–radio delay might then reflect the time evolution of the phenomenon which produced the electromagnetic burst.

Also, the duration δt of the radio burst, even in the case of the lowest frequency 151 MHz, cannot be attributed to dispersion across the bandwidth of the receiver. In fact, if the full 60-s delay between the γ-ray burst and a truly prompt radio burst were caused entirely by electron dispersion, δt would be only 0.8 s for a bandwidth $\Delta\nu = 1$ MHz.

Our radio data seem to be the radio counterpart of August 16, 1976, γ-ray burst. The probability of a random coincidence (8×10^{-5}) could have been drastically reduced by other independent observations. Unfortunately, this was not the case. We hope that more extended collaborations between various spaced stations will increase the efficiency in rejecting interference events and give further evidence of the existence of radio bursts of cosmic origin.

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Observational test for the existence of a rotating black hole in Cyg X-1

X-RAY satellite experiments[1,13,14] are now investigating the degree and plane of linear polarisation of the radiation from Cyg X-1. This radiation can be explained as coming from an accretion disk around a black hole, the polarisation of the X rays being due to electron scattering in the hotter, inner regions of the disk. Novick reported[2] observing 3% linear polarisation (marginally significant at the 2.5σ level) at 2.6 keV from Cyg X-1. Existing predictions of the polarisation properties, as a function of energy, have been based on a Newtonian approximation[3-5], thus neglecting gravitational effects on the rays as they propagate from the disk's surface to an observer at infinity We present here preliminary results of a full general-relativistic calculation which show that gravitational effects completely alter the polarisation properties, and provide a sensitive test of the existence of a black hole.

Whereas in the Newtonian approximation the plane of polarisation as measured at infinity is the same for each point of origin on the disk (for the same initial polarisation), it varies considerably when we allow for general-relativistic effects. These rotational differences can amount to over 100°. Even the special-relativistic aberration contributions, in the regions of interest, are typically an order of magnitude smaller than those of a gravitational origin.

We use the general-relativistic parallel-transport equations[6] to propagate the polarisation vector from each point on the disk along the null geodesic linking it to an observer at infinity with polar angle θ_0. While a non-rotating, Schwarzschild, black hole can be treated analytically (R. F. Stark, unpublished work and ref 7), we report here numerical results for a Kerr black hole. For a rapidly rotating black hole the general-relativistic effects on the polarisation properties are an order of magnitude greater than for a slowly-rotating black hole or for a neutron star.

The degree of linear polarisation, δ, of the rays as they leave the disk will also differ from the Newtonian value. Gravitational bending of the light will alter the angle at which a ray leaves the disk's surface from the Newtonian value of θ_0. As δ depends on the initial direction of travel (varying, for an optically thick disk, from 0% for rays leaving the disk vertically, up to 12% for rays leaving parallel to the disk)[9], we can also expect gravitational effects to change the observed degree of polarisation.

We sum contributions from all parts of the disk to find the net observed polarisation properties, as a function of observed X-ray energy E_0, from the net Stokes parameters $\bar{I}(E_0), \bar{Q}(E_0), \bar{U}(E_0), \bar{V}(E_0)$ (ref. 8). Following Cunningham[9], we use a transfer function f to obtain the intensity, but also define a polarisation transfer function $h = fe^{2i\varphi}$ where φ is the observed angle of the plane of polarisation (measured from the unrotated direction). The net Stokes parameters can then be expressed as

$$\bar{I}(E_0) = \iint G(g)\, f\, I(E_0/g)\, r\, \mathrm{d}r\, \mathrm{d}g$$

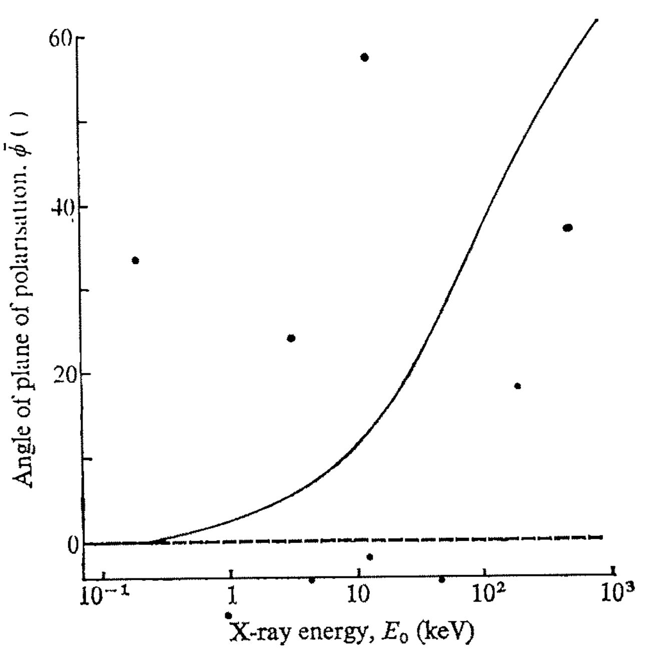
$$\bar{Q}(E_0)+\mathrm{i}\bar{U}(E_0) = \iint G(g)\, h\, \delta\, (E_0/g)\, I(E_0/g)\, r\, \mathrm{d}r\, \mathrm{d}g$$

where G is a function only of the redshift g, and I is the locally produced intensity. The assumption of a plane, homogeneous atmosphere for the disk implies that $\bar{V}(E_0) = 0$, that is, there is no circular polarisation. The net degree of polarisation, and net polarisation angle, $\bar{\delta}(E_0)$, $\bar{\varphi}(E_0)$, as observed at infinity are then given by:

$$\bar{\delta}(E_0) = \surd\, [\bar{Q}^2(E_0)+\bar{U}^2(E_0)]/\bar{I}(E_0),\ \tan 2\bar{\varphi} = \bar{U}(E_0)/\bar{Q}(E_0)$$

For illustration, we have used the Novikov–Thorne co-rotating, equatorial disk model[10], for a Kerr black hole with specific angular momentum $a/m = 0.9981$ (ref. 11) although, as we emphasise, and discuss below, we expect similar effects with most disk models centred on rapidly rotating black holes. For our chosen parameters the disk could always be considered to be optically thick throughout. We use Chandrasekhar's results[6] for the variation of δ with angle for such an atmosphere,

Fig. 1 Variation of plane of polarisation with energy for $\theta_0 = 75.5°$. The parameters used for the disk model were: viscosity parameter, $\alpha = 0.1$, mass of black hole, $M = 9M_\odot$; mass accretion rate $= 7\times10^{17}$ g s^{-1}. ———, General-relativistic curve; — — —, Newtonian curve.



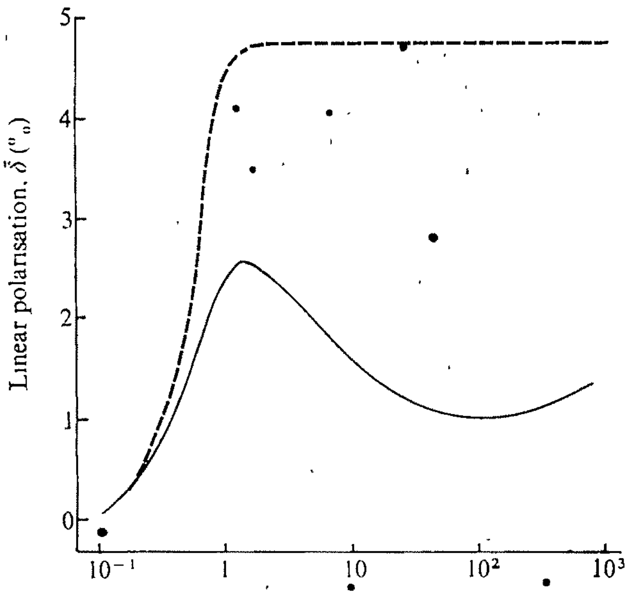


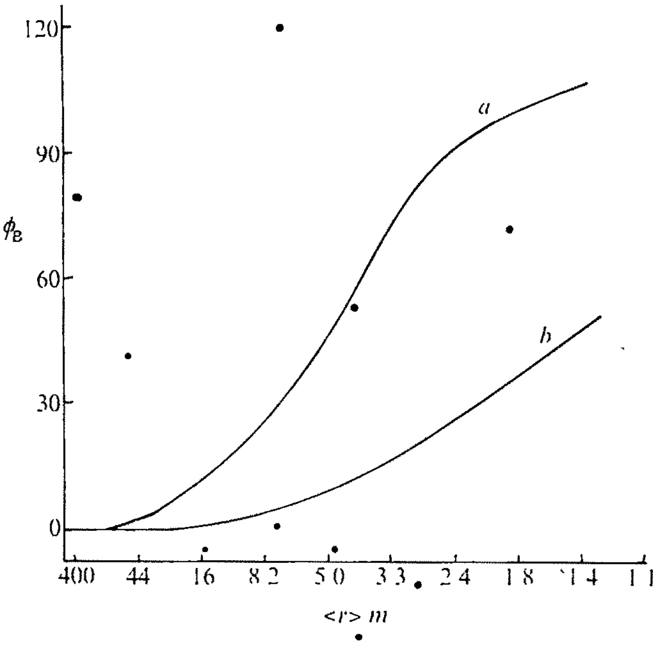
Fig. 2 Variation of linear polarisation with energy. Same parameters as in Fig. 1. ———, General relativistic curve; — — —, Newtonian curve.

modified to include frequency-dependent effects due to free–free opacity.

Figure 1 shows the large general-relativistic variation of the polarisation plane with energy. An anti-clockwise rotation with increasing energy will be seen by an observer above the disk (that is, with $\theta_0 < 90°$), while observers below the disk will see a clockwise rotation. The effect of general relativity on the degree of polarisation (Fig. 2) is to cause a peak (below the predicted Newtonian value) and a fairly rapid fall-off at ~2 keV, and to cause a minimum and subsequent rise at higher energies. This behaviour is predominantly due to the gravitational bending of the rays previously mentioned, the depolarisation due to differential rotations in φ being smaller.

A large general-relativistic rotation in the plane of polarisation can be expected for most other disk models incorporating electron scattering, and a good estimate of the rotation for these models can be found by examining the peaking behaviour of the integrals with redshift and radius. For a chosen model, as E_0 increases, I peaks for an annulus of decreasing radius, on account of the radial temperature structure of the disk. Around this annulus the redshift factor G peaks where the rays leaving it are blueshifted the most into E_0. We thus find that the main contribution to the integrals comes from a small area of the disk. Figure 3 shows the variation of the observed polarisation plane φ_B of these maximum blueshifted rays with annular radius $\langle r \rangle$.

Fig. 3 Variation of the polarisation plane of the most blueshifted radiation with emission radius. $a/m = 0.9981$. a, $\theta_0 = 41.1°$, b, $\theta_0 = 75.5°$.



Once the variation of $\langle r \rangle$ with E_0 for a particular model has been found, the variation of φ_B with $\langle r \rangle$ can be used to obtain a good estimate for φ. If the disk has an optically thin inner region, $\bar{\varphi}$ will change by 90° as $\langle r \rangle$ passes through the transition region from thick to thin[12], and will then resume a general-relativistic variation. Large variations in $\bar{\varphi}$ with energy, of a gravitational origin, will occur whether the inner region is optically thick or thin (as in the two-temperature disk model)[15].

The very large general-relativistic rotations in the plane of polarisation provide an opportunity for testing the black hole hypothesis for Cyg X-1. To observe these effects, X-ray satellite experiments are required with more sensitive polarimetry across a wider energy range than is available at present. The results also indicate that the polarisation properties of radiation emitted from regions of strong gravitational fields can be both theoretically and experimentally important.

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X rays from radio binaries

THE properties of the radio binary systems, CC Cas, AR Lac, β Per (Algol), β Lyr, b Per and Cyg X-1 have been listed[1]. A thermal interpretation of the radiation from Algol[2] requires a much larger X-ray flux than the observed value of 3.8×10^{-11} erg cm^{-2} s^{-1} keV^{-1} in the 2–6-keV energy range[3]. The observation of some non-thermal flares, and the small size of the radio source in Algol indicate the non-thermal nature of the radio emission[1]. We interpret the radio emission as synchrotron radiation and suggest that the observed X-ray emission is inverse Compton scattering of the light of the primary star by the radio electrons. We calculate X-ray emission from other radio binaries using this model. The energy for the radio electrons can arise from annihilation of magnetic lines connecting the binary stars twisted by the rotation of the stars[4].

The energy of the synchrotron electrons of β Per (Algol) is fixed by the optical photon energy (about 0.8 eV) and the X-ray photon energy (4 keV); the relativistic factor for these electrons is about 75. The observed radio frequency (about 8×10^9 Hz) gives the strength of the magnetic field as 1.6 G in the radio-emitting region, using the usual sychrotron radiation formulae. The radio emission from the electrons, with the observed radio energy emission[1], gives the number of radio electrons as 2×10^{38}. The optical luminosity of

TABLE 1 X-ray flux from radio binaries

	CC Cas	AR Lac	β Per	β Lyr	b Per	Cyg X-1
Optical luminosity (erg s^{-1})	5×10^{36}	2×10^{34}	3×10^{35}	6×10^{36}	2×10^{35}	5×10^{36}
Maximum radio luminosity at 8085 MHz (erg s^{-1} MHz^{-1})	1.2×10^{19}	7.3×10^{17}	4.8×10^{17}	1.6×10^{18}	3.9×10^{16}	2.6×10^{20}
Distance (pc)	1000	71	25	260	52	2500
Calculated X-ray flux (erg cm^{-2} s^{-1} keV^{-1})	2×10^{-12}	10^{-12}	4×10^{-11}	3×10^{-11}	10^{-12}	10^{-11}
Observed X-ray flux (erg cm^{-2} s^{-1} keV^{-1})	?	?	3.8×10^{-11}	?	?	$\sim 4 \times 10^{-9}$

the primary B-star is calculated from the observed visual magnitude and an appropriate bolometric correction, and is about 10^{36} erg s^{-1} The optical photon density is obtained using a distance of 3×10^{11} cm from the star, which is a little less than half the semi-major axis of the close binary system Using a distance of 25 pc for Algol[1], the X-ray intensity at the Earth due to Compton scattering of the optical photons from radio electrons is about 4×10^{-11} erg cm^{-2} s^{-1} keV^{-1}, agreeing with the value of Schnopper *et al* [3]

The energy release, E, from the magnetic field twisting between the binary stars is given approximately for one turn as (see ref. 4)

$$E \sim B_S^2 a^6/R^3 P \text{ erg s}^{-1}$$

where B_S is the surface polar magnetic field of the star, a is the radius, $2R$ is the interstar distance and P is the period of rotation Using plausible values of $B_S \sim 30$ G, $P \sim 10^5$ s, $a \sim 2 \times 10^{11}$ cm, $R \sim 4 \times 10^{11}$ cm, we obtain $E \sim 10^{31}$ erg s^{-1}, which is approximately the energy in X-ray emission The value of B_S is compatible with the magnetic field in the radio-emitting region if we assume a dipole field.

Table 1 shows the X-ray flux expected from the other radio binaries listed in ref. 1 using the same model The X-ray flux observed from Cyg X-1 clearly shows that the X-ray-emitting process is different from the one considered here. Of the remaining radio binaries in the list, β Lyr seems the most promising candidate for observation

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Transient structure in the high-energy X-ray light curve of NP 0532

ON 1974 June 21 we observed the Crab Nebula with a balloon-borne X-ray telescope (20–150 keV) with a sensitive area of ~ 700 cm^2. The observations took place between UT 54112 s and 78520 s. At times during this period a transitory third peak was observed in the light curve of NP0532, which we have reported earlier[10]. The peak comprised $2.0 \pm 0.5\%$ of the total emission from the Crab, and was detectable for ~ 5 min on two occasions (~ 40 min apart). We also report pulsed fractions in the primary and secondary peaks, and in the interpulse region.

Four light curves for the pulsar NP0532 are shown in Figs 1*a*–*d* (35–115 keV). The intervals over which data were superimposed are indicated. A fifth curve (Fig. 1*e*), is a superposition of background data, using the period for NP0532. The pulsar period was corrected for balloon location and drift, based on optical data and calculations provided by H. Helmken of the Center for Astrophysics. The data were binned in time bins of 1/85 of the pulsar period, that is, ~ 0.3901 ms. The actual time resolution of our data, however, is 2 ms.

Fig. 1 Light curves of NP0532 on 21 June 1974 (35–116 keV). The observation intervals (in UT; seconds from June 21.0) are indicated. Scans over the Crab Nebula were made with different exposures; thus, the amplitudes of the peaks are different for the four intervals: *a*, 54130–54800 s; *b*, 71880–72200 s; *c*, 73700–74200 s; *d*, 74450–74850 s. The primary and secondary peaks occupy bins 25–45 and 66–81, respectively. A third peak (bins 5–12) is apparent ~ 12 ms before the primary in *b* and *d*. A curve for background data (*e*, 81600–82600; folded at the NP0532 period) is also given.

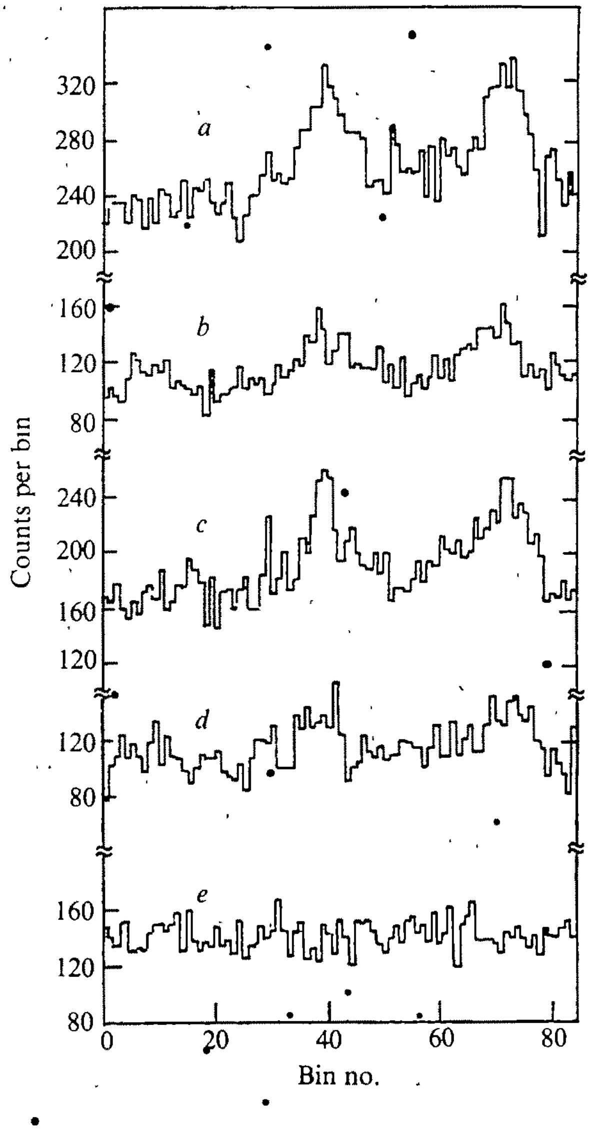

Table 1 Analysis of transient light-curve peaks

Bins per group	No. of groups	χ^2_{max}		Starting bin for χ^2_{max}		Null hypothesis probability	
		b	*d*	*b*	*d*	*b*	*d*
6	4	16.6	13.3	5	3	0.0036	0.0169
7	4	15.5	10.3	6	2	0.0063	0.0680
8	3	19.2	9.9	5	3	0.0002	0.0392
9	3	13.6	17.0	6	4	0.0093	0.0013
10	2	12.7	9.0	5	3	0.0020	0.0196
11	2	15.1	8.8	5	3	0.0010	0.0215
12	2	14.1	11.5	5	3	0.0020	0.0066
13	2	8.3	11.0	5	2	0.0306	0.0074
14	2	6.4	8.5	5	14	0.0775	0.0295

Bins 1–24 and 82–85 in the light curves of Figs. 1*b* and *d* were used. The first column gives the number of time bins combined into larger bins (called 'groups'). The second column gives the number of groups that could be composed from the data. The third and fourth columns give the maximum value of χ^2 obtained by forming groups from adjacent time bins from Figs 1*b* and *d*, respectively. This maximum occurred when the first of these groups began with the bin number given in the fifth column (Fig. 1*b*) or sixth column (Fig. 1*d*). The sixth and seventh columns give the probability that the given values for χ^2_{max} for the data of Figs 1*b* and *d*, respectively, would occur as statistical fluctuations in random data. These probabilities were determined by repeating the analysis procedure on 10,000 runs of computer-generated random data.

Figures 1*b* and *d* show the presence of a third pulse (bins 5–13) which leads the primary pulse by ~12 ms. To establish objectively a level of statistical significance for this peak, we used a Monte Carlo technique, that is, we tested for a feature of arbitrary location and width within the light curve. In applying this technique, we first calculated χ^2 for various combinations of adjacent data bins (which we called 'groups') from Figs 1*b* and *d*; a total of 28 bins (bins 1–24 and 82–85) were used in constructing the groups, covering the generally accepted 'unpulsed' portion of the light curve. Groups consisting of 6–14 bins were considered; the lowest-numbered bins were used and leftover bins disregarded when 28 was not divisible by the number of bins per group (for example, when considering groups of six bins, only bins 1–24 were used). χ^2 was evaluated for these groups, fitting the data to a constant count rate. The maximum value of χ^2 obtained in this manner is given in Table 1 for each group size used.

The same procedure was then followed on 10,000 runs of simulated data, generated assuming a constant counting rate equal to the mean observed rate and fluctuations dictated by Poisson statistics. The number of runs (divided by 10,000) which produced values of the maximum χ^2 in excess of that obtained with the actual data is noted in Table 1 for each group size. These numbers are, therefore, the probabilities for a constant source producing the values of χ^2_{max}. For a group size of eight bins, only two out of the 10,000 runs of simulated data produced as large a value of χ^2 as did the data from Fig. 1*b*. Consequently, we conclude that it would be very unlikely for the Crab pulsar, if truly constant over these bins, to produce the observed data. Note that this probability level does not directly give the statistical significance of any peak present; a 'valley', for example, could as easily produce high values of χ^2 in the simulated data. The significance of the feature in Fig. 1*d* is considerably less, as 13 runs (out of 10^4) yielded larger values of χ^2 (group size of nine bins).

The width of the apparent peak (eight or nine bins) is consistent with a much narrower feature, broadened by our time resolution; of course, it is not necessarily any narrower than eight or nine bins. We were able to rule out a single, brief burst (duration ~ a few ms) as the cause of the feature. We subdivided the 320 s of data used in Fig. 1*b* into two 160-s subintervals and established that each subinterval contributed approximately equal amounts to the feature. A duration somewhat greater than the ~5 min we report is also possible, as we did not observe the Crab during the ~4 h between the times when data for Figs 1*a* and *b* were taken. The feature was not evident ~20 min later, however, in a scan beginning at UT 73280 s.

We also established the pulsed fraction of the total Crab spectrum from our data, as a function of energy, by combining ~2 h of data taken between UT 54150 s and 78380 s on 21 June 1974 (assuming a photon number spectrum of $22E^{-2.29}$ photons cm^{-2} s^{-1} keV^{-1} for the total Crab flux[7]). Over such a long interval, the transient third peak washes out. Consequently, for this ~2-h interval, we report pulsed fractions only for the primary peak, the interpulse region, and the secondary peak (Table 2). The unpulsed portion of the light curve is comprised of the sum of 28 bins: 1–24 plus 82–85.

Table 2 Pulsed fractions in X-ray emission from Crab (nebula and pulsar) (21 June 1974, 2 h data taken between UT 54150 s and 78380 s)

Bins	Designation	% total flux over given energy range 20–28	28–35	35–47	47–64	64–80	80–115	115–146
25–45	Primary peak	4.5±0.8	5.6±0.9	5.5±0.6	5.6±0.5	6.8±0.6	5.9±0.8	6.7±1.7
46–65	Interpulse region	3.4±0.7	2.7±0.8	3.4±0.6	4.1±0.4	3.9±0.6	4.9±0.8	4.6±1.6
66–81	Secondary peak	5.9±0.7	7.0±0.8	6.0±0.5	6.6±0.4	6.9±0.6	9.0±0.7	7.2±1.4
Σ	Total pulsed	13.8±1.7	15.2±1.9	14.8±1.4	16.3±1.0	17.6±1.4	19.9±1.8	18.6±3.7

For the period of time during which the third peak was most evident (Fig. 1*b*), we calculated its contribution to the flux from the Crab by assigning only the sum of bins 1–4 plus 13–24 plus 82–85 to the 'unpulsed' portion of the light curve. We find that it comprised a fraction

$$f_t = 0.020 \pm 0.005$$

of the total Crab flux (35–115 keV).

The possibility of time variability in the NP0532 X-ray light curve was first suggested by Boldt *et al.*[1], based on a factor of 1.5–2 difference between their sounding rocket measurements of the pulsed fraction in 1968 (2–10 keV) and those of Bradt *et al.*[2] in 1969 (1.5–10 keV). Additional peaks in the NP0532 light curve at approximately the same location as the third peak in Figs 1*b* and *d* have been reported[3,12]. Large, infrequent variations on time scales of a few tenths of a second have been reported[4] in the 2–6 keV X-ray flux from the immediate vicinity of the Crab, based on *Uhuru* data. Helmken[6] has pointed out an apparent discontinuity in the light curve, obtained by subtracting the NP0532 optical light curve (normalised to the X-ray primary peak) from a composite X-ray light curve, at a point

~10 ms preceding the primary peak. He suggests a possible association with the third peak (Figs 1*b* and *d*) reported here.

For an assumed isotropic radiation pattern, the energy radiated in the third peak would be $\sim 4\times10^{37}$ ergs (at an assumed source distance of ~2 kpc) This is likely to be an overestimate, since the Earth probably lies near the plane of the pulsar rotational equator[9]. It is interesting to compare this number to the energy associated with pulsar spinups (and subsequent spindowns). For the September 1969 spinup, the fractional change in the pulsar rate has been given as $|\Delta\omega/\omega| \sim 3\text{–}9\times10^{-9}$ (refs 5, 8). Scargle and Pacini[11] argued that the accompanying total energy release from the pulsar magnetosphere was $\sim 10^{43}$ erg The associated rotational energy gain of the rotator would have been $\sim 10^{41}$ ergs If a similar scaling relationship were to apply for the transient third peak reported here, the resulting value for $|\Delta\omega/\omega|$ would be $\sim 10^{-14}$. Even if the pulsar spinup energy were comparable with the energy radiated as X rays, $|\Delta\omega/\omega|$ would still only be $\sim 10^{-12}$, far below the level of detectability.

In summary, the third peak is transient in nature; its duty cycle seems to be low ($\lesssim 10\%$). Further observations of this phenomenon will require extended measurements with detectors of even greater sensitivity than the ones we used.

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Diffusion time scales and accretion in the Sun

SURFACE abundances in the Sun could have been essentially caused by accretion, either of comets or of grains[1–3]. It has been hypothesised that if surface convection zones could be smaller than is usually indicated by model calculations, then accretion could be especially important. Dust accretion by the Sun has also been suggested as the cause of ice ages on the earth[4]. Either the zone immediately below the surface convection zone is stable enough for diffusion to be important, however, or other transport processes (turbulence, meridional circulation) more efficient than diffusion, will tend to homogenise the star. Diffusion is the slowest of the transport processes and will become important when the other transport processes become inoperative. Using diffusion theory, we can determine the minimum mass of the convection zone, if transport processes at the bottom of the convection zone are not to influence the abundances in the convection zone. If diffusion time scales are shorter than the life of the star, diffusion will modify the abundances in the convection zone. The mass in the convection zone for which diffusion time scales are equal to the life of the star on the main sequence then determines the minimum mass in the convection zone to justify neglect of transport processes at the bottom of the convection zone. I determine here that, for the Sun, this mass is between 3×10^{-3} and $10^{-2}M_\odot$, and give a general expression for the diffusion time scale as a function of the mass of the convection zone.

Within the convection zone, the turbulent velocities are large enough to ensure homogeneous abundances[5]. The surface abundances are assumed to be the same as those in the convection zone. If the convection zone is considered to be mixed and emptied through its bottom then letting Y be the mass fraction, in the convection zone, of the element of interest

$$\frac{\mathrm{d}}{\mathrm{d}t}(Y\Delta M_c) = Yw_i\rho. \qquad (1)$$

ΔM_c is the mass of the convection zone in g cm^{-2}, w_i the diffusion velocity and ρ the density at the bottom of the convection zone (w_i negative towards the solar interior). Equation (1) integrates to give

$$Y = Y_0 \exp(-t/\theta) \qquad (2)$$

with

$$\theta = -\Delta M_c/\rho w_i \qquad (3)$$

Values of θ have been obtained[6] in seven models of main sequence stars of mass $1\text{–}2.6M_\odot$. In the Sun, most heavy elements will diffuse downwards, unless the mass in the convection zone is $< 10^{-6}M_\odot$. Gravitational settling dominates in solar type stars. Aller and Chapman[7] made similar calculations for the Sun, but neglected radiation pressure and used an incorrect thermal diffusion term (see ref. 8 for discussion). For helium in the Sun, one obtains, from equations (37), (8), and (10) of ref. 6

$$\theta = 3.6\times10^{5}\,\Delta M_c^{0.545}\ (\mathrm{yr}) \qquad (4)$$

or

$$\theta = 2.3\times10^{11}\,\Delta M^{0.545}\ (\mathrm{yr}) \qquad (4a)$$

In equation (4), ΔM_c is in g cm^{-2} and in equation (4a), ΔM is the total mass in the convection zone in solar mass units. For helium in the Sun, equation (4) is exact to within a few per cent. For $\Delta M = 3\times10^{-3}M_\odot$, $\theta \simeq 10^{10}$ yr, and $\theta \simeq 1.7\times10^{10}$ for $\Delta M = 10^{-2}M_\odot$. The diffusion time scales for manganese are, in the Sun, nearly equal to those of He (see Table IV of ref. 6), but those of Hg are approximately 40% smaller. For transport processes to have negligible effects on surface abundances in solar type stars, the mass in the convection zone must be at least 3×10^{-3} to $10^{-2}M_\odot$. In solar models with the mixing length equal to the pressure scale height, the mass in the convection zone is $2\times10^{-3}M_\odot$.

Use of equation (4) to estimate diffusion time scales for helium in main-sequence stars lighter than $2M_\odot$ is accurate to within a factor of 1.5, but leads to a factor of three overestimate in θ below the hydrogen convection zone of a $2.6M_\odot$ star.

Diffusion time scales in the Sun are very uncertain. The uncertainty arises from our poor knowledge of ΔM_c, that is from the hydrodynamics. Equation (4) is much better known than is ΔM_c. If we use this uncertainty on ΔM_c to increase the relative importance of the accreted mass, then if $\Delta M \leqslant 3\times10^{-3}M_\odot$, transport processes at the bottom of the convection zone must be taken into account, and the convection zone will lose the accreted matter, through its bottom. The calculations of ref. 3 suggest that $\Delta M \leqslant 5\times10^{-4}M_\odot$ for accretion to be important. For $\Delta M = 5\times10^{-4}M_\odot$, diffusion reduces Z, in the convection zone, from 0.02 to 0.01 A more generous estimate of the accreted mass is given by ref. 2, and for an upper limit of $5\times10^{-3}M_\odot$, diffusion would reduce Z by approximately a factor of 0.7. At the lower limit of ref. 3, ($\Delta M = 5\times10^{-5}M_\odot$), Z in the convection zone would be reduced from 0.02 to 0.002 or less. Transport processes at the bottom of the convection zone do not rule out completely the possibility that heavy elements in the convection zone, and so at the surface, were accreted by the Sun, but make it much less likely.

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Planetary tides during the Maunder Sunspot Minimum

CONTROL of the 11-yr and longer cycles of solar activity tides raised on the Sun resulting from the gravitational pulls of the planets has often been suggested. Rudolf Wolf and R. C. Carrington were among the early astronomers who pointed out this possibility in the middle nineteenth century[1]. The close coincidence of the sidereal period of gravitationally important Jupiter (11.87 yr) with the mean period of the observed annual sunspot means (11.3 yr) raises the possibility of such a relationship; the range of possible configurations of the other planets allows a wide realm of other tidal periods and effects. In daily sunspot numbers, a small but consistent periodicity at the sidereal period of the planet Mercury has been found[2], and a possible 178.7-yr period in sunspots (about twice the Gleissberg cycle) has been linked with multi-planet tidal influences[3]. Wood and Wood[4] have applied a dynamical theory which includes all planets but Mars to reinforce their belief in a more than chance relationship. We reconstruct here Sun-centred planetary conjunctions and tidal potentials for the AD 1645–1715 period of sunspot absence (the Maunder Minimum). These are found to be effectively indistinguishable from patterns of conjunctions and power spectra of tidal potential in the modern era of a well-established 11-yr sunspot cycle. This places a new and difficult constraint on any tidal theory of sunspot formation.

The relative tide-raising forces of the planets are as follows: Mercury 0.91, Venus 2.15, Earth 1.0, Mars 0.03, Jupiter 2.26 and Saturn 0.11, with the three outer planets negligibly small. Problems arise in any direct gravitational theory because of the apparently insufficient forces and tidal heights that are involved: the tide-raising force of Jupiter on the solar photosphere is, for example, about 10^{12} times smaller than the force of solar gravity there, making planetary tides a miniscule force[1], even in the case of a rare, nine-planet conjunction. Thus proponents of the tidal hypothesis usually revert to trigger mechanisms, which is difficult to criticise or, apparently, to test by observation.

Any tidal theory rests on the evidence of continued sunspot periodicity, and the substantiation of a prolonged period of solar anomaly in the historical past[5,6] offers a possible *experimentum crucis* The Maunder Minimum was the most drastic change in the behaviour of solar activity in the last 300 yr, in that sunspots almost disappeared for a 70-yr period (1645–1715), and the 11-yr cycle was probably absent. During that time, however, the nine planets were all in their orbits, and planetary conjunctions and tidal potentials were indistinguishable from those of the modern era, in which the 11-yr cycle is well-established. This provides good evidence against the tidal theory

We have pressed the case further by reconstructing the pattern of planetary tidal forces during the Maunder Minimum to investigate the possibility that the multiple-planet forces somehow fortuitously cancelled at the time, that is, that the positions of the slower moving planets in the late seventeenth and early eighteenth centuries were such that conjunctions and tidal potentials were at the time reduced in number and force.

Historical planetary positions were determined using a computer code supplied by R. S. Harrington of the U.S. Naval Observatory and the mean elements of planetary orbits determined by Seidelmann *et al.*[7]. Only Mercury, Venus, Earth and Jupiter were considered in final analysis because the tidal forces generated by combinations with other planets are at least an order of magnitude less than these. A conjunction was defined as a Sun-centred alignment of planets within 0.15 rad, to assure that Mercury would remain in such an alignment for > 1 d.

Daily positions were calculated from J.E.D. 2296445 to 2451065 (about AD 1575–2000) and all two-, three-, and four-planet conjunctions noted. The results are shown in Fig. 1, with the history of the annual mean sunspot number. Binary conjunctions are omitted in the presentation. Higher order conjunctions exhibit subtle and beautiful symmetries and beat patterns, but there is no striking dissimilarity between the time of the Maunder Minimum and any other period shown.

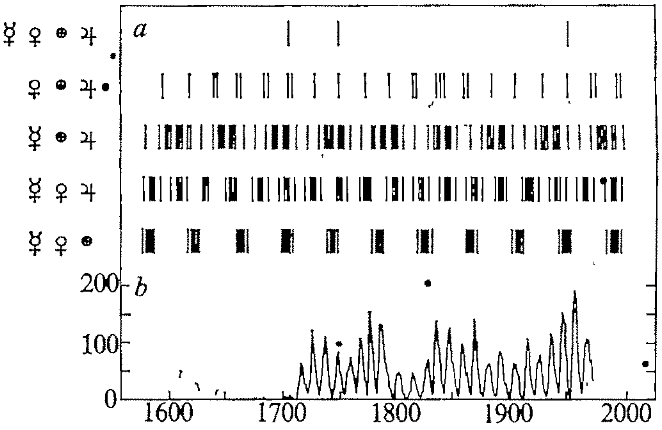

Fig. 1 *a*, Multiple planetary conjunctions (alignment within 0.15 rad) AD 1575–2000. *b*, Mean sunspot number[8].

The failure of planetary conjunction patterns to reflect the drastic drop in sunspots during the Maunder Minimum casts doubt on the tidal theory of solar activity. Yet this test is only a qualitative judgement of a pattern and not demonstrably conclusive. A more quantitative test comes from an examination of the tidal potentials, which are proportional to

$$P(\mathbf{x}) = \sum \frac{M_i}{R_i^3}\left(\cos^2\varphi_i - \frac{1}{3}\right) \qquad (1)$$

where M_i is the mass of planet i, R_i is its distance from the centre of the Sun, and φ_i the angle, seen from the centre of the Sun, between the planet and the point $\mathbf{x}$.

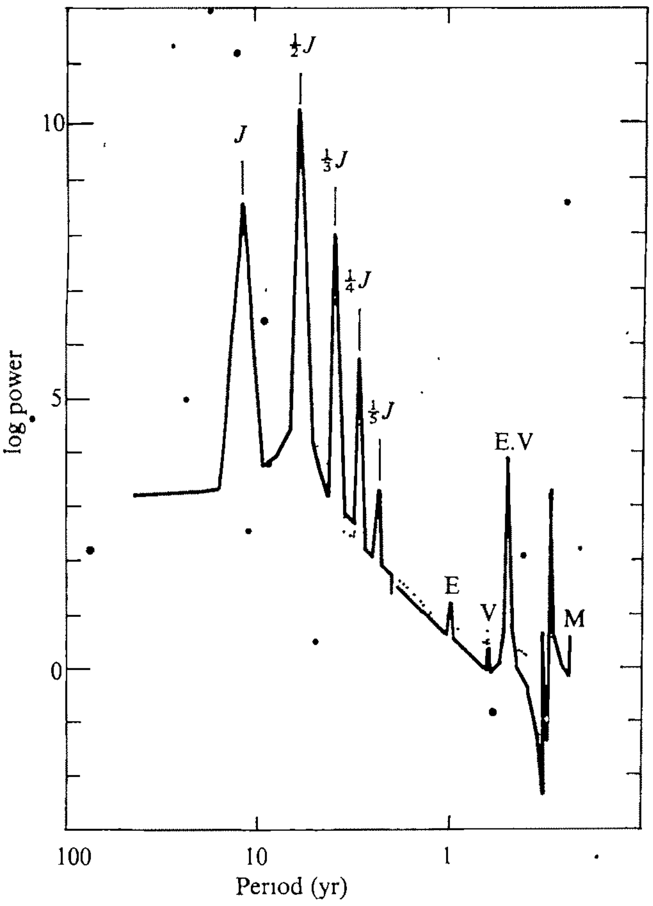

Fig. 2 Fifty-year power spectra of the tidal potential of planets on the rotating equator of the Sun (equation (1)), ———, AD 1925–1975; ....., which appears only where different from heavy line, AD 1650–1700. No differences appear in the major tidal peaks or in any other significant feature of the spectrum for these or any other epochs we examined. Major peaks correspond to fundamental and harmonic periods of the major tide-producing planets, identified by letter: J, Jupiter, E, Earth; V, Venus; M, Mercury.

We calculated a daily value of the tidal potential defined above at a point fixed on the rotating equator of the Sun, for AD 1450–2000. The period was then divided into sample intervals of about 50 yr, overlapped at 25-yr intervals, and the power spectrum of tidal potential calculated for each 50-yr sample using a fast Fourier transform. An example of the power spectrum of tidal potential is shown in Fig. 2. Principal peaks in the spectrum correspond to orbital periods of each planet and the harmonics thereof. (L. Auer has pointed out that each harmonic corresponds to the frequency of an epicycle in a Copernican decomposition of the orbit.) The usual means of dealing with the functional periodicity implied by Fourier techniques, such as tapering the end points to the mean, were inadequate in this case, since the longest periods of interest are a large fraction of the 50-yr sampling interval. This problem was avoided by adjusting the length of the sampling interval so that the potential function and its derivative were equal at the end points, hence the implied periodic function was continuous.

To compare the power spectra of tidal potential in different intervals we selected as a standard interval the modern period AD 1925–1975 (Fig. 2) and computed the ratio of the spectrum of this standard interval to all others from AD 1450 to 2000. We found that the power in each of the major peaks of tidal potential (notably the dominant first three harmonics of Jupiter) have not changed perceptibly in the last 500 yr. Also, there was no appreciable change in the power at any frequency during the years of the Maunder Minimum. In Fig. 2 we compare power spectra for the 1925–1975 period with that of the Maunder Minimum and find them effectively indistinguishable.

A few exploratory calculations showed possibly significant changes in the spectrum of tidal potentials over much longer periods (several millennia) which seem associated with known, secular changes in the orbital elements of the planets. For example, the eccentricity of Jupiter's orbit changes by about 0.1% per century[7]. There is no evidence, however, that these secular changes are associated with possible secular changes in solar activity.

Okal and Anderson[8] in examining the same problem computed a single power spectrum of the planetary tidal potential (or rather, its maximum value in longitude) for 10-d intervals from roughly AD 1800 to 3600. This sample, much longer than ours, defined the frequency of power peaks rather precisely. They found only one peak near that of the sunspot cycle, at the orbital period of Jupiter, 11.86 yr. They compared this with a power spectrum estimate on Wolf sunspot numbers[9] which showed peaks at 11.0 and 9.8 yr in this region, and reasoned that the discrepancy between 11.86 and 11.0 yr sufficed to rule out tides as a driving force for the solar cycle.

Peaks in the maximum entropy spectra derived by Cohen and Lintz[9] vary with the details of the input function: drastically in amplitude, and less in frequency. Their published examples suggest that the method of computation and data selected can shift the location of peaks near 11.0 yr period by at least ±0.1 yr, and amplitudes by a factor 3. This is consistent with the difficulties with spectral decomposition by the maximum entropy method[10,11]. Moreover, the period chosen by Cohen and Lintz includes all sunspot numbers from 1750 onward. Waldmeier[12] casts doubt on the quality of the early sunspot data as does Eddy[6], who points out that controlled observation of sunspots did not commence until about 1852. Thus, since the power peaks derived by Cohen and Lintz were based in part on questionable data, the significance of an apparent discrepancy between these sunspot power peaks and those of averaged tidal periods seems less than conclusive, and in need of the support presented here.

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Order and disorder in weakly ionised plasmas

WE report the observation of novel radial structures for discharges in pure helium and argon gases in a magnetic field. The structure depends on the angle between the discharge tube axis and the magnetic field direction, a dependence which supports the idea, previously proposed, that gaseous plasma energised in specific conditions can have regions in which the electrons have an ordered spatial distribution.

The weakly ionised, high-frequency, electrodeless discharges were energised at powers between 500 W and 1 kW coupled to the discharge from an amplifier via a 143-cm long quarter-wavelength twin transmission line; this was connected to two copper bands, 14 cm apart, placed closely around the outside of the discharge tube. The discharge was maintained continuously between the bands and in a position centrally between a pair of Helmholtz coils in a horizontal, uniform magnetic field.

Optical flats sealed to each end of the hard glass tubes made it possible to view the discharges axially. The tubes were 70 cm long, and of internal radius 1.84 cm. A pattern of luminous and non-luminous plasma regions was observed, arranged in coaxial symmetry, the complexity of the pattern depending on the magnitude of **B**. At **B** = 0 (Fig. 1*a*) shows the simplest pattern for helium gas at 0.25 torr pressure. At **B** = 1,000 G, (Fig. 1*b*) besides the outer dark shell, a central non-luminous plasma was observed, the outer luminous region now being enclosed between the two dark regions. At **B** = 1750 G the inner dark region was larger in diameter, and a further plasma region, this time luminous and blue in colour was seen on the tube axis, inside and much shorter in length than the inner dark region (Fig. 1*c*).

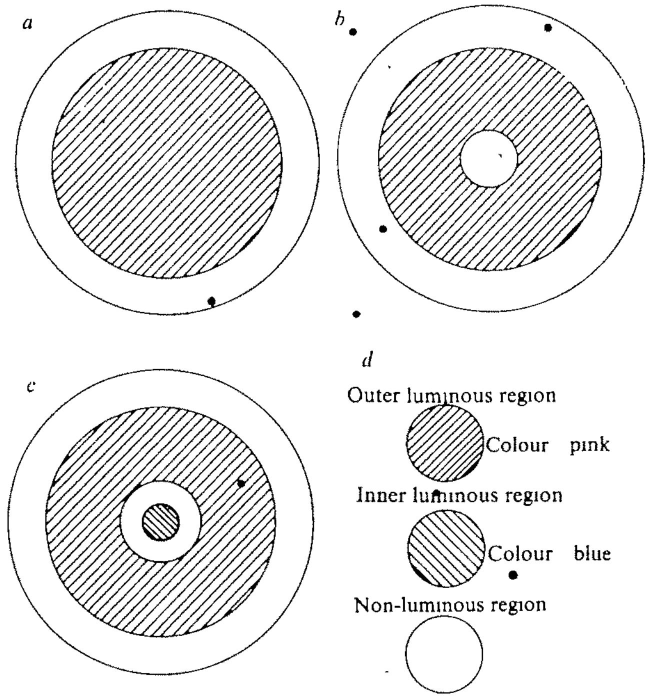

Fig. 1 Plasma regions: *a*, **B** = 0; *b*, **B** = 1,000 G; *c*, **B** = 1,750 G; *d*, spectral analysis of helium light and key to figures.

Visible lines identified

Wavelength (nm)	Transition
438.8 (w)	$5^1D \rightarrow 2^1P$
443.8 (w)	$5^1S \rightarrow 2^1P$
447.1 (s)	$4^3D \rightarrow 2^3P$
471.3 (s)	$4^3S \rightarrow 2^3P$
492.2 (s)	$4^1D \rightarrow 2^1P$
501.6 (s)	$3^1P \rightarrow 2^1S$
504.8 (s)	$4^1S \rightarrow 2^1P$
587.6 (vs)	$3^3D \rightarrow 2^3P$
667.8 (w)	$3^1D \rightarrow 2^1P$
706.5 (vw)	$3^3S \rightarrow 2^3P$

w = weak
vw = very weak
s = strong
vs = very strong

Figure 1*d* gives an analysis of the spectral emission from the outer visible region. The calculated electron temperature for this region is 85,000 K, and the measured average electron density for all regions was 7×10^{10} cm^{-3}.

Experiments in which the helium discharge tube was suspended from a spectrometer base and rotated in the horizontal plane showed that at given field strength, the diameters of the central regions were critically dependent on θ, the angle between the tube longitudinal axis and the direction of **B**; this is shown in Fig. 2*a*, in which the diameters are plotted against θ for **B** constant in magnitude (**B** = 1,750 G). Figure 2*b* shows how the diameters of both inner and outer regions vary with the field strength, keeping θ = 0. Given the results of Fig. 2*a*, it follows that there is a critical value for θ, θ_c, for which the central regions disappear or reappear as θ is increased or decreased, respectively, at fixed **B**. Figure 3 is a plot of θ_c against **B**.

In argon gas, for the range of field strengths of the experiment, the central regions differ from those for helium. In argon at 0.37 torr pressure, for example, no luminous central region is observed, and the dark central region is just beginning to appear at **B** = 1,650 G.

The central plasma structure (Fig. 1 *b* and *c*) and its dependence on θ, (Fig. 2*a*), are significant evidence for the presence of order in the non-luminous regions. These regions are believed to be occupied by oscillating electrons which have very little energy and are therefore not capable of exciting gas atoms, so that the regions are non-luminous. The basic theory of the interaction between low energy electrons and large numbers of gas atoms and molecules in which they are immersed, was given by Fermi[1]. According to his analysis, in which the electrons are treated as electron waves, stationary states form under certain conditions. For the present purpose we assume that the oscillators are formed from pairs of electrons, or aggregates of pairs of electrons, and the requisite conditions for stationary states relate the electron number density, *n*, the neutral atom

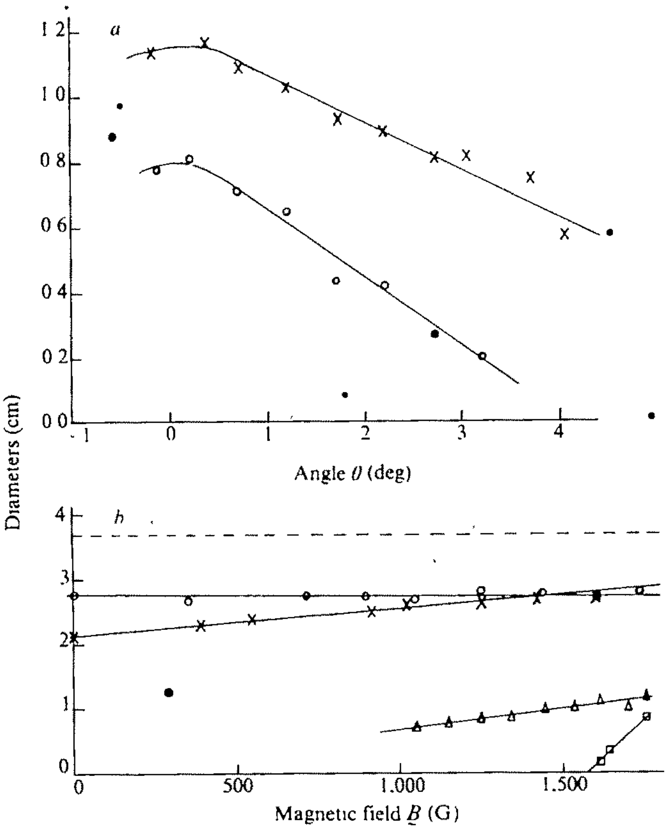

Fig. 2 *a*, The diameters of the central regions against θ (**B** = 1,750 G) ×, Non-luminous region. ○, Luminous region. *b*, The diameter of the plasma regions against **B** (θ = 0). — — —, Tube internal glass wall diameter, ○, diameter of the outer region junction (0.25 torr helium); ×, diameter of the outer region junction (0 67 torr argon), △, diameter of the inner non-luminous region (0.25 torr helium); □, diameter of the inner luminous region (0.25 torr helium).

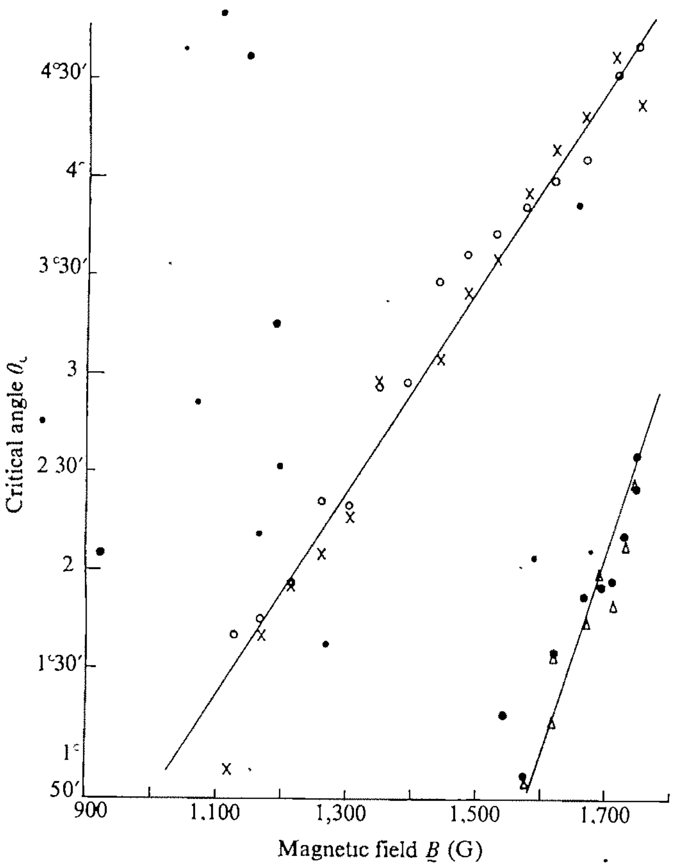

Fig. 3 The critical angle, θ_c against **B** (central regions). ×, Non-luminous region (θ increasing); ○, non-luminous region (θ decreasing); ●, luminous region (θ increasing); △, luminous region (θ decreasing).

number density, N, and atomic constants[2]. When $\mathbf{B} = 0$ at low pressures the conditions occur near the tube walls, where n is smaller than on the tube axis; when **B** is raised to > 1000 G, however, the magnetic energy of the oscillators becomes significant and the conditions for stationary states are changed. In helium, the conditions are found not only near the tube walls but also at higher electron densities on the tube axis where the energies of oscillations are higher. A non-luminous region then appears on the tube axis. In argon, on the other hand, the potential energy of the interaction of the oscillating electrons and the gas atoms is negative rather than positive as in helium[1], a difference between the gases which results in the differences in the variation of the outer dark shell radius with **B** (Fig. 2*b*), and in the central structure. These are the lines on which an interpretation of the phenomena is believed to be possible.

Both the relatively sharp boundaries between the luminous and non-luminous regions, and the dependence of the central structure on θ are considered to show that the oscillators form a close-packed matrix, capable of reflecting fast-moving free electrons from the luminous regions, and of having anisotropic properties typical usually of crystalline substances.

The idea that ordered plasmas are sometimes formed in weakly ionised gases was prompted, not by the present visual studies, but from a prolonged study[3] of the properties of rare gas afterglow plasmas. From these studies we inferred a division of the afterglow plasma into two regions for $\mathbf{B} = 0$; at the start of electron density decay, we deduced that the junction of the two regions should be near the point on the tube radius where n has fallen to one half of its value on the axis. Assuming a simple Bessel function variation of n with radial position, this prediction from the afterglow work is that the junction between the two outer regions should occur near a diameter of 2 29, cm (in good agreement with Fig. 2*b* for $\theta = 0$).

Other measurements can be interpreted successfully using the model; for example, the measurement of forces of weak elastic constraint acting on plasma electrons[4], the very fast plasma decay rates observed at $\mathbf{B} = 0$, the anisotropic properties of afterglows in a magnetic field[5], and the resonances attributable to electron spin-flip in a magnetic field, can all be interpreted in terms of the ordered electron model. The anisotropic properties of afterglow plasmas are good evidence of correlation between the afterglow phenomena and the currently studied continuously excited plasmas. An examination of the value for θ required for the transition from slow to fast afterglow decay rates in helium at 0.25 torr pressure as a function of **B** reveals that when θ is just large enough for the fast decay rate to occur, the central dark plasma region in the excited plasma preceding the afterglow is just disappearing (Figs 2*a* and 3).

It is interesting to consider an extension of the idea of spatially ordered electron oscillators to some naturally occurring plasmas. Such an extension needs a change of emphasis in the theory, away from electrons as particles translating with random motion in the space between the other plasma particles, to electrons as waves occupying and propagating through the space containing the ionic, atomic and molecular fields.

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Metal accumulation rates in sediments from Mid-Indian Ocean Ridge and Marie Celeste Fracture Zone

THE existence of metalliferous sediments on mid-ocean ridges is well documented[1] and the general conclusion is that they form by precipitation from submarine hydrothermal solutions. Pacific and Atlantic Ocean sediments have been studied in detail[2–6]. We describe here material from the Indian Ocean Ridge, collected during cruise 8/75 of RRS Shackleton, and compare metal accumulation rate data for ridge-crest and -flank sediments with those from other regions. The metalliferous sediments are present on the Mid-Indian Ocean Ridge, particularly near the Triple Junction[7,8], and Bostrom and Fisher[7] suggest that "there is a complete belt of iron-rich sediment along the entire length of the Indian Ocean–Pacific Ocean active-ridge system". We have found ferromanganese encrustations on basalts from the median-valley wall and have recognised what are probably sulphides in decorated vesicles of basalts. The metal accumulation rate data argue against a hydrothermal source of metals in the ridge sediments, however, and suggest that metalliferous sediment accumulations in the Indian Ocean may be more local phenomena than previously realised.

Sample locations and bathymetry of the study area are shown in Fig. 1. The Marie Celeste Fracture Zone is sharply defined by topography, seismicity and magnetics[9] and is one of the larger fracture zones offsetting the Mid-Indian Ocean Ridge. The half-spreading rate of the ridge is ~ 1.8 cm yr^{-1} and the

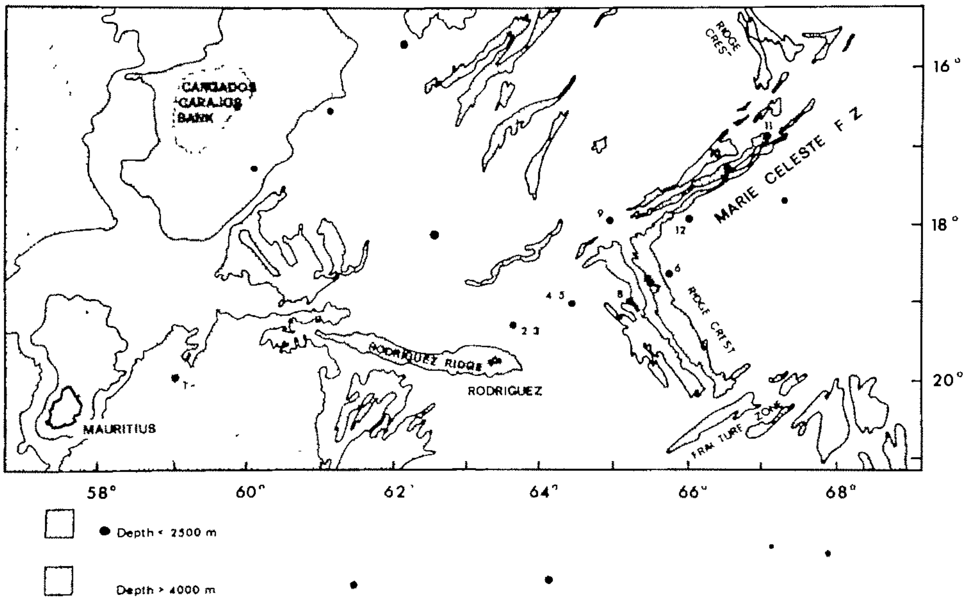

Fig. 1 Core location map showing bathymetry in region of Marie Celeste Fracture Zone. ●, Cores; ■, manganese crust.

ridge offset is 220 km (ref. 9). The maximum depth of the fracture zone is reported as 5836 m (ref. 10). The region shown in Fig. 1 seems to be a likely location for hydrothermal solutions and associated metal-rich sediments[5].

The sediment type ranges from siliceous lutite of low to moderate carbonate content, to calcareous lutite (~ 90% $CaCO_3$) in relation to the depth of deposition. Physical parameters (texture, colour, etc.) are very similar between and within the carbonate cores, suggesting that the three cores studies in detail are representative of the remainder. One core collected from the fracture-zone floor consists of siliceous lutite overlain by 110 cm of red clay. Chemical and microscopic comparisons with the carbonate sediments suggests that the fracture-zone core represents a condensed sequence deposited below the carbonate lysocline.

Table 1 Typical analyses of ridge-crest and flank sediments and sediment from the Marie Celeste Fracture Zone

%	GC2 (0–2 cm)	GC4 (0–2 cm)	GC7 (8–9 cm)	GC10 (0–5 cm)*	GC12 (0–2 cm)
SiO_2	1.75	1.58	1.61	18.0	3.18
TiO_2	0.024	0.029	0.022	0.32	0.040
Al_2O_3	0.41	0.40	0.47	4.6	0.60
Fe_2O_3	0.58	0.72	0.62	5.92	1.31
MnO_2	0.093	0.119	0.094	0.71	0.178
MgO	0.41	0.37	0.68	2.71	0.52
CaO	50.9	50.6	49.7	31.3	48.2
K_2O	0.080	0.089	0.093	0.53	0.113
P_2O_5	0.10	0.22	0.13	—	0.22
Na_2O	1.36	0.90	1.71	3.62	1.40
L.O.I.	42.93	42.59	42.48	30.78	41.27
Total	98.61	97.57	97.62	98.49	97.06

*From the fracture-zone floor. SO_4, Cl not determined.

There is no significant change in major-element chemistry with depth, except as a consequence of a 3–5-cm glass horizon which occurs between 37 and 62 cm in individual cores, and the compositions listed in Table 1 are typical of these sediments. The volcanic glass is acidic (refractive index ~ 1.50) and fine grained, and probably derives from Indonesia (G. Walker, personal communication, 1976). Also present are basalt fragments containing decorated vesicles of a type already reported[11–13]. Figure 2 shows a scanning-electron micrograph of a vesicle revealed by the fracture of a basalt fragment during sample preparation. Such decorations have been interpreted as sulphides[11–13] and their occurrence in the sediment is supported by evidence of sulphide mineralisation in Indian Ocean ridge basalts[14].

Mean sedimentation rate was obtained by radiocarbon dating of a section of core 12 at 18–20 cm which gave an age of approximately 20,000 yr. The rate of ~ 1 cm 10^{-3} yr^{-1} was confirmed by oxygen isotope stratigraphy (N. Shackleton, personal communication, 1976) and is in agreement with other estimates and measurements for this region [10,15,16] and with world averages for carbonate sedimentation[17]. Metal accumulation rates (Table 2) were calculated by age correlation using the acidic glass horizon. Comparison with accumulation rates in normal pelagic sediments and in hydrothermal sediments from the East Pacific Rise shows that metals are accumulating at these locations on the Mid-Indian Ridge at rates commen-

Fig. 2 Decorated vesicles in a basalt fragment from a carbonate core. The wall decorations are probably sulphides.

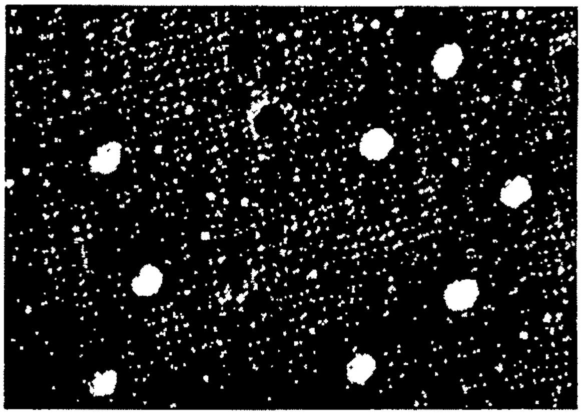

Table 2 Element accumulation rates (mg cm^{-2} 10^{-3} yr^{-1}) in Indian Ocean, normal pelagic, and East Pacific Rise sediments

	Indian Ocean Marie Celeste Fracture Zone	Ref. 23	Pelagic sediments Ref. 18	Ref. 24	East Pacific Rise sediments Ref. 1	Ref. 3
Ti	0.08–0.2	0.2–0.9	—	0.4	0.074	—
Al	1.5 –2.6	4–18	—	6	0.72	—
Si	4.2 –9.6	—	—	17	—	55
Fe	2.4 –5.2	3–9	—	4	63	110
Mn	0.4 –0.7	0.3–1.0	0.2–3.2	4	24	35

surate with the lower rates of pelagic sedimentation. Hence, no unusual processes are responsible for metal deposition in these sediments. The deposition rate for manganese (0.4–0.7 mg cm^{-2} 10^{-3} yr^{-1}) is similar to that in nodules (0.2–1.0 mg cm^{-2} 10^{-3} yr^{-1}) (ref. 18). A slow, uniform precipitation of Mn over the sea floor has been inferred from the similar rates of Mn accumulation in nodules and sediments from all oceans[18] and suggests that the metals accumulating on the Mid-Indian Ridge are authigenic in origin. The ratio Al/Al+Fe+Mn for these sediments (0.24–0.35) is, however, within the range used to classify Mid-Indian Ridge sediments as metal rich and of volcanogenic origin[1,7,8]. The sediments we examined are not of volcanogenic origin although they conform to this classification. It is clear that metal–aluminium ratios do not necessarily indicate regions of metalliferous sediment deposition and must be used with caution in this respect.

Despite the extensive nature of hydrothermal deposits on the East Pacific Rise many metal-rich deposits are of a very localised nature[2,5,6,19–21]. Our results do not disprove the existence of metal-rich exhalations and deposits within the area studied, but suggest that such deposits on the Mid-Indian Ridge are probably local and certainly less widespread than sometimes supposed. We made many attempts to sample sediment from within and on the flanks of the median valley in the hope of detecting hydrothermal material. No sediment was recovered, however, but, instead, the corer sampled a fragmented ~ 15 mm thick ferromanganese encrustration on basalt.

The encrustation contains 20.0% Fe, 12.5% Mn, 0.11% Cu, 0.081% Co, 0.14% Ni and 0.060% Zn. For manganese to be accumulating at a rate $\gg$ 1 mg cm^{-2} 10^{-3} yr^{-1} the oceanic crust at this point must be $\ll$ 120,000 yr old. As the encrustation was recovered from the median valley wall this is possible and it is just conceivable that this material may contain a component of local hydrothermal origin. Its overall composition, however, is unlike that of hydrothermal manganese crusts from the Mid-Atlantic Ridge median valley[6], The Galapagos Spreading axis[2] or the Romanche Trench[5], and is very similar to S.W. Indian Ocean nodules[22]. In the absence of other evidence, therefore, it must be regarded as a hydrogeneous deposit.

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Estimation of gas-releasing efficiency of erupting magma from ^{226}Ra–^{222}Rn disequilibrium

VOLATILE components of volcanic magma have an important role in its explosive eruption[1]. The volatiles may be partially released from the erupting magma into the air, during eruptive events, and the ratio of the released to the original amount may be a possible determinant of the mode of explosive eruption. Direct measurements of these quantities, however, are extremely difficult; fresh ejecta are rarely obtained. The Sakurajima Volcano, Kyushu, Japan, erupted explosively on 30 October 1975, and discharged pumices and ash-like materials, some of which were later accessible. We report here a change in the measured radioactivity of pumice, possibly due to radon release.

Both types of ejecta were collected immediately after the eruption by the staff of the Sakurajima Volcano Observatory, Kyoto University. Within 48 h these samples were subjected to non-destructive γ-ray spectrometry, using a Ge(Li) semiconductor detector, at the Radioisotope Centre, University of Tokyo, to determine their contents of natural short-lived radionuclides (Table 1).

Gamma-ray spectrometric data so far obtained for some of the products from major eruptive episodes of the Sakurajima show no significant change in the U- to Th-series ratio of magma for the last few hundreds of years (Sato and Sato, in preparation). These data are included in Table 1 for comparison. Compared with the 'ash' sample and historical lavas, the 1975 pumice sample showed a marked deficiency in ^{214}Bi. A measurement performed on the same sample 1 month later showed that the specific activity of ^{214}Bi increased to a value similar to those for other materials listed in Table 1; the ascendant short-lived nuclides in the U-series which had been depleted through the eruptive event could have increased during the period. This observation agrees with the differing origins of the two types of ejecta: the pumice represents fragments of the erupting magma whereas the ash-like material could be composed of fragments of rocks pre-existing around the crater.

The significant degree of vesiculation of the pumices shows that a substantial amount of gases was discharged. The gaseous radioelement, radon, may have been expelled at this stage of eruption. One of radon's isotopes, ^{222}Rn (half life: 3.8 d), requires about 1 month to increase to an

Table 1 Th- and U-series radioactivity in some products of Sakurajima Volcano (10^{-13} Ci g^{-1})

Sample	Th-series (from ^{228}Ac and ^{208}Tl)	U-series (from ^{214}Bi)	U-series / Th-series
Pumice, 30 October 1975; andesite*	6.7±0.8	1.84±0.61	0.28±0.09
'Ash', 30 October 1975; andesite*	5.7±0.2	3.8±0.2	0.67±0.04
Lava, 1914; andesite	5.7±0.2	4.4±0.3	0.77±0.06
Lava, 1776; andesite	8.5±0.7	5.6±0.5	0.66±0.08
Pumice, Moeshima; dacite	5.7±0.2	3.8±0.2	0.67±0.04

*Measurements were made within a few days of their fall.

amount in equilibrium with the parent, ^{226}Ra, and is expected to show its transient deficiency for several days after the event. This could explain the lowering of the U- to Th-series ratio observed for the pumice (Table 1). The ratio is, therefore, regarded as being indicative of the ^{226}Ra–^{222}Rn disequilibrium.

In subsequent eruptive activities (a detailed account will be published later), the volcano ejected, on 13 May 1976, a considerable amount of pumice. Figure 1 shows U- to Th-series radioactivity ratios for this material, and shows that the U- to Th-series ratio of the 1976 pumice was again significantly lowered for several days after the eruption, then increased to a value close to those of earlier lavas (Table 1).

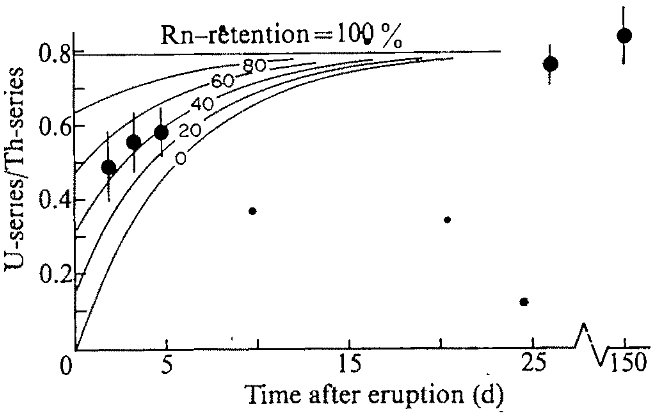

Fig. 1 Growth of U- to Th-series radioactivity ratio observed for the 1976 pumice from Sakurajima. Growth curves are based on the assumption that a ^{226}Ra–^{222}Rn equilibrium was established in the magma before the eruption. The values are plotted against the mid-points of counting times.

The disequilibrium observed above may also be due to radon release at the moment of eruption. If a ^{226}Ra–^{222}Rn equilibrium is assumed to have been established in the magma before the eruption, the growth of ^{222}Rn in the pumice should indicate its re-establishment. The data are expected to fit a growth curve derived from two parameters: (1) the observed U- to Th-series activity ratio of pumice at the equilibrium and (2) the percentage of unreleased radon in the erupting magma. In Fig. 1 are shown the growth curves for postulated values of (2), and, on this basis, the data correspond to a 50–60% release of radon.

The behaviour of radon in an erupting magma may be different from that of major volatiles. It would be possible, however, to characterise explosive eruptions by extending the method presented here to other fresh pyroclastic materials.

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Effects of inbreeding on cognitive performance

THE few studies[1,2] in which the effects of inbreeding on cognitive performance have been examined revealed that offspring of first-cousin marriages had lower IQ scores than offspring of unrelated parents. These studies were, however, performed in societies where the population engaging in such marriages is a small (1%, 6%)[2,3] and unrepresentative proportion of the total population. Possible confounding of the effects of inbreeding with the effects of other intelligence-related variables such as socioeconomic status may lead the effects of inbreeding to be overestimated[2] Unfortunately statistical control may either over- or undercorrect for the correlates of the independent variables, leaving one in doubt about the true effect of inbreeding. I have now examined the effects of inbreeding on cognitive performance in an Arab population with a high rate of consanguinous marriage which minimised the distortions due to non-genetic variables. I show here that offspring of unrelated parents performed better than offspring of first-cousin marriages in intelligence and achievement tests administered at grades 4 and 6 The lowest level of performance and a higher variance were found for offspring of double-cousin marriages The inbreeding depression found in this study is consistent and cannot be explained by the effects of socioeconomic status. I drew a nationally representative sample of 3,203 children in grades four and six (approximate ages 10 and 12 yr) of the Arab educational system in Israel This sample constitutes about 10% of the total population in these grades and includes only normal (not retarded) children. Column 1 in Table 1 shows the division of the subjects according to grade level and consanguinity of the parents. A first-cousin marriage is between children of siblings. Children of first cousins have, on average, 1/16 pairs of genes by common descent. Double first cousins are children of siblings married to unrelated siblings. When they marry, their children have, on the average, 2/16 pairs of genes by common descent.

A study of Arab education in Israel revealed that the frequency of consanguinous marriages in the Arab population is about 34%. The Arab community encourages such marriages and therefore the group that intermarries should be a representative sample of the population Double first-cousin marriages are hardly known in Western Society but exist to a significant degree (about 4%) in my sample population. Their popularity is no doubt partly explained by economic considerations concerning the custom of 'bride price' When a brother and sister of one family marry a brother and sister of another, there is no need for the groom to pay the bridal price.

It is reasonable to assume that other consanguineous marriages—between, for example, second and third cousins —exist in our sample. However, only three alternatives were given to the question on blood relationships (unrelated, cousins and double cousins) and, consequently other consanguineous marriages were probably classified by the respondents as one of the two latter categories, especially first cousin marriages. This would tend to make the real inbreeding coefficients somewhat lower than the above coefficients (1/8, 1/16). We can also assume that, since prolonged inbreeding increases the coefficient of inbreeding[5], the inbreeding coefficient of the control group is not zero. But prolonged inbreeding has also occurred for the inbred groups, so it is safe to assume that there exists an increasing order of the degree of inbreeding, from the control group (of outbred children) to the first cousins' offspring and to the double cousins' offspring.

Table 1 Consanguinity and socioeconomic status of subjects

	Number of subjects	Father's education (mean yr)	Mean SES†
Grade 4			
First cousins	503	5.6	9.7
Double first cousins	71	5.1	9.1
Others (unrelated)*	1,054	5.2	9.4
Total sample	1,628	5.3	9.5
Grade 6			
First cousins	467	5.4	9.5
Double first cousins	54	5.0	9.1
Others (unrelated)*	1,054	5.0	9.4
Total sample	1,575	5.1	9.4

*In a population with a high rate of consanguinous marriages the so-called 'unrelated' group probably does not have a zero inbreeding coefficient (see text).

†Socioeconomic status.

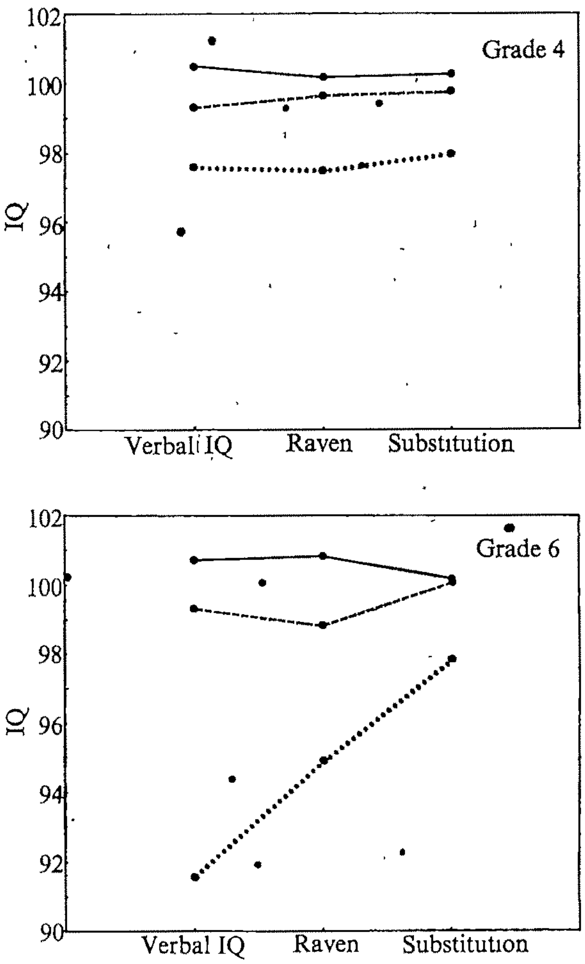

Fig. 1 Mean IQ scores on the three mental ability tests to parental consanguinity, for grades 4 and 6. ——Not related, - - - cousins, ... double cousins.

Socio-economic status (SES) of the groups was defined as an index of the following four components. (1) father's plus mother's level of education; (2) father's profession; (3) room density, and (4) physical home conditions. Each of the four components was divided into four graded categories. SES was defined as the sum of these four components, thus ranging from four to sixteen, with each of the four components being of equal weight. Table 1 shows that unrelated parents have somewhat lower SES than the first cousins. The double first cousins have lower SES than the unrelated group but the difference is less than 0.1 s.d. As the very small differences in SES favour the first cousins as compared with the other two groups, any reduction in performance found in the inbred groups cannot be explained by differences in SES.

Three mental ability tests and four achievement tests were used. As the genetic component is assumed to be larger in informal learning (ability tests) than in specific knowledge formally taught in the classroom (achievement tests) I expected inbreeding to have a smaller effect on performance in achievement tests than in ability tests. The mental ability tests included (1)*a*, a verbal general knowledge and comprehension test and *b*, a verbal test to find the 'odd one out'. Both tests were equally weighted to construct a single verbal IQ score (Alpha reliability coefficient is 0.93, correlations averaging 0.70 with achievement tests and teacher's evaluation) (2) A shortened version of Raven's Progressive Matrices[6] was used to measure nonverbal ability (3) A substitution test that, unlike the other two, was given under a strict time limit and was used to measure associative learning. The four achievement tests were constructed in accordance with the specific school curricula of each grade (Arabic, Hebrew, mathematics and science). The alpha reliability coefficients range from 0.80 to 0.91 and the correlations between the four tests average about 0.70. All tests were administered to groups of children and given simultaneously in all the sample classes.

Table 2 presents the mean scores and standard deviations for the mental ability and achievement tests, according to grade and parental consanguinity. Figure 1 shows the IQ scores on the three mental ability tests for the different consanguinity groups, separately for each grade. Figure 2 shows the *t* scores of achievement tests (with a mean of 50 and s d. of 10 for each test).

All the results follow the expected pattern and the order of performance level of the three groups is consistent. Outbred children achieved the highest level of performance and offspring of double cousin marriages achieved the lowest. Thus, an inbreeding depression is suggested in both grades and in all the tests.

The results in Table 2 show a tendency towards higher variance in the double cousin group. This is the case in 13 out of the 16 possible comparisons; the result is statistically significant in five of the comparisons, all of which are in grade 6 (general knowledge, finding the odd one out, Arabic, Hebrew and science).

The inbreeding depression shown in Table 2 and Figs 1 and 2 is too modest to warrant changes in educational policy, but the consistency of the effect for the different tests in both grades strengthens the argument that its cause is indeed genetic. Surprisingly, there was no difference between performance in achievement and ability tests: inbreeding

Table 2 Mean scores and standard deviations of mental ability and achievement tests according to parental consanguinity and grade level

		Mental ability tests								Achievement tests							
		Verbal ability															
Grade	Parental consanguinity	General knowledge (51)*		Finding odd-one-out (25)*		Raven's Matrices (28)*		Substitution (144)*		Arabic (58/57)†		Hebrew (51/51)†		Mathematics (39/40)†		Science (29/30)†	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
4	'Unrelated'	32.2	8.3	12.8	5.6	8.8	5.1	53.5	24.4	37.1	11.9	21.8	7.8	21.2	7.9	14.7	5.1
	First cousins	31.5	8.9	12.3	5.8	8.6	5.1	52.7	28.2	36.3	12.0	21.3	7.9	20.8	8.4	13.5	5.3
	Double first cousins	31.0	9.3	11.4	5.9	7.9	5.1	49.6	23.7	34.4	12.9	21.1	8.1	20.1	8.9	13.3	5.3
6	'Unrelated'	38.4	8.3	16.4	5.9	13.1	6.1	70.5	27.0	37.9	10.6	31.4	10.2	18.6	8.7	17.6	5.7
	First cousins	37.6	8.2	15.8	6.1	12.3	6.2	70.4	27.0	37.4	10.7	30.4	10.3	18.3	8.5	17.3	5.7
	Double first cousins	33.0	12.0	13.0	7.3	10.6	6.7	65.8	27.0	32.0	13.2	27.7	12.4	16.9	10.0	15.2	7.1

*Number of items in the test

†The left-hand numbers represent the number of items in the test for grade 4 and the right-hand numbers, number of items for grade 6.

depression seems clear and consistent in both. It is interesting that the effect is smaller for the mathematics and substitution tests, though the three groups fall into the same order here as for the other tests.

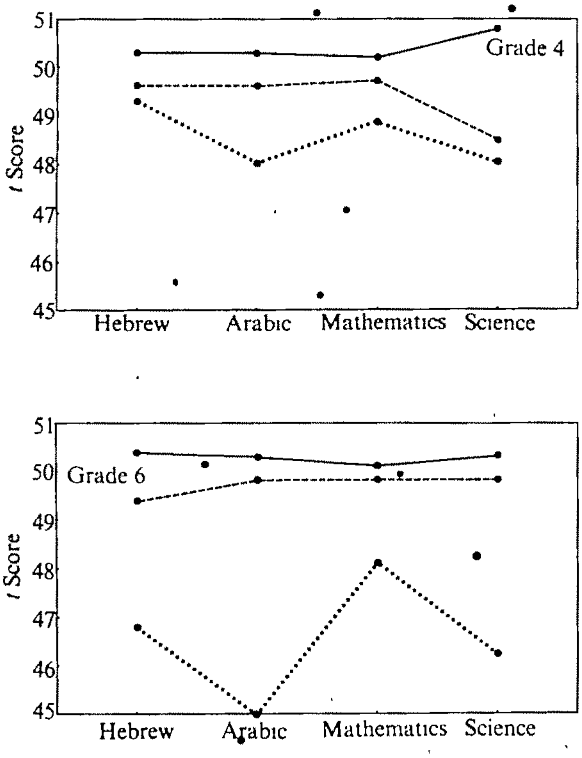

Fig. 2 Standardised means (t scores) of achievement tests according to parental consanguinity for grades 4 and 6 ——, Not related; – – –, cousins, ..., double cousins,

The inbreeding depression clearly demonstrates the importance of a genetic component in the variance of cognitive performance in this population This could result either from the general increase in homozygosity that results from inbreeding or from decrease in performance resulting from homozygosity for specific recessive alleles[7,8]. The higher variance of the double cousin group in some of the tests favours the second interpretation.

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The significance of the nose for certain phenomena of visual perception

BOWER[1] draws attention to the fact that the majority of organisms have evolved in such a way that part of the body (almost invariably the nose) is permanently present in the visual field. This, as he points out, can hardly be accidental and indicates that the organ may have a function in visual perception; subjects lacking a nose may differ from normals on tests of visual capacity. Unfortunately this clinical condition is now rarely encountered and affected individuals may, in any case, develop compensatory techniques, for example, reliance on glimpses of some other part of the body. It may be more profitable to consider the effects of making the nose visible where this is not normally the case. The autokinetic phenomenon may, for example, be a consequence of the fact that in total darkness the nose is invisible and cannot play its customary role as a stable point of reference by which other objects in the visual field are judged stationary or in motion with respect to the observer. What would be the effect of making the nose visible? I report here two experiments in which the nose was visible in an otherwise dark visual field. Contrary to Bower, autokinetic movement seems to have persisted.

The autokinetic phenomenon occurs when a subject fixates a point source of light in an otherwise dark field. The light characteristically appears to move, though it is in fact stationary throughout. Recent experimental work has vastly increased our understanding of the parameters governing the extent of this phenomenon, but none of the proposed explanations has survived experimental test. Bower's proposal implicating the nose is prima facie appealing but has not been confirmed empirically. What is required is a means of making the nose visible to the subject in an otherwise dark visual field since movement should not then be reported. Two alternative methods suggest themselves. The subject may wear a luminous false nose, or small electric light bulbs may be inserted in his nostrils illuminating the nose from within Each method has disadvantages. The luminous false nose has a pale greenish-white appearance and does not correspond in size and conformation to the subject's own nose (being usually somewhat larger). The inserted light bulbs involve a degree of physical discomfort but the organ visible is the subject's own, even though translucent pink in colour. Both methods were used in the two experiments to be reported. The luminous false nose condition presented no special problems of method but in the inserted light bulb condition it was found that because the conformation of the nostril and the thickness of the nasal membrane differ from subject to subject, a greater or lesser area around the nose was sometimes illuminated To ensure that no additional visual cues were available, the subject was enveloped from the neck down in a voluminous cloth of black, non-reflective material similar (except in colour) to those used by hairdressers.

In Experiment 1 each subject spent three 2-min periods in total darkness fixating the point source and reported the onset and offset of apparent movement if any occurred. Ear-muffs were worn to avoid interference from irrelevant auditory stimuli The subject was allowed 5 min to adapt to the darkness before beginning his first observation period and rested for 3 min in dim illumination between periods

Each subject did one period wearing a luminous false nose, one with light bulbs inserted in his nostrils and one control period. A standard moulded rubber artificial nose was used, coated with non-toxic luminous paint The nostril illuminator consisted of a pair of 6-V, 0.36-W L.E.S bulbs partially embedded in plastic. These were disinfected between subjects using 5% cetrimide solution. Twelve subjects were tested (all female, average age 18.56 yr). In order to equalise the effects of practice and fatigue the three experimental conditions were presented to two subjects in each of the six possible orders of presentation

The results obtained are shown in Table 1. Three subjects did not report movement in any condition, results are therefore tabulated for nine subjects only

Table 1 Incidence of autokinetic movement in Experiment 1

	Illuminated nostril	Control	Luminous nose
Time of onset (s)	38.22	47.44	28.11
Duration (s)	41.67	38.44	55.78

There is no significant difference between the illuminated nostril condition and the control condition, either in time of first onset measured from the beginning of the period ($t=0.25$, not significant), or in duration—that is, total time during which the point of light was in (apparent) motion ($t=0.28$, n s.) The luminous false nose condition seems to have enhanced the effect (onset, $t=1.65$, n.s., duration, $t=2.32$, $P<0.05$).

It might be argued that the control condition in Experiment 1 was inadequate, since although the subject was unable to see his nose he was also free of the physical sensations and possible discomfort associated with the experimental conditions (particularly the illuminated nostril condition). In a second experiment each experimental condition was compared directly with a control condition in which the subject wore an ordinary (non-luminous) false nose or in which light bulbs were inserted in the nostrils but were not illuminated. Twenty-four subjects were tested in all four conditions (12 male, 12 female, average age 18.67 yr) The four experimental conditions were presented once each in the 24 possible orders of presentation. Procedure was otherwise as in Experiment 1.

The results obtained are shown in Table 2. No effects of sex were observed All subjects reported movement in at least two conditions

Table 2 Incidence of autokinetic movement in Experiment 2

	Illuminated nostril	Non-illuminated nostril	Luminous nose	Non-luminous nose
Time of onset (s)	41.33	27.37	25.71	24.58
Duration (s)	49.50	61.50	59.00	61.92

The luminous false nose condition did not affect the phenomenon, but the illuminated nostril condition seems to have been associated both with later onset and with shorter duration of apparent movement. Unfortunately, these differences (which would have supported Bower's hypothesis) fail to reach significance by the t test (onset, $t=1.71$, n.s.; duration, $t=1.53$, n.s.).

It may be that the techniques employed were not sufficiently refined. (We experienced some difficulty in persuading subjects unfamiliar with the requirements of strict experimental method to sit in total darkness, wearing ear-muffs, with electric light bulbs inserted in their nostrils —particularly when the bulbs were not actually illuminated). But the project is at an early stage. The results so far obtained while not positively refuting Bower's hypothesis, cannot be said to support it.

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Sampling theory for alleles in a random environment

A MAJOR effort in theoretical population genetics has been aimed at developing statistical tests to distinguish between various explanations of observed patterns of enzyme variation[1]. Perhaps the most useful result to emerge from this effort is Ewens' sampling theory of neutral alleles[2]. This theory allows one to use the number and configuration of alleles in a sample to test for departures from neutrality. Watterson[3] has derived the analogous sampling distribution for a finite population subject to the joint effects of mutation and symmetric heterotic selection. It differs from the neutral sampling theory, providing a stronger hypothesis-testing situation than has previously been available. In this note I present a third sampling distribution. It applies to an infinite population in which the selective coefficients fluctuate at random through time and space. Quite unexpectedly, the sampling distribution turns out to be identical to that for the neutral model, indicating that agreement of data with Ewens' sampling distribution does not rule out the possibility that natural selection is responsible for the genetic variation in the sample. Furthermore, the properties of random-selection populations at equilibrium are indistinguishable from neutral-allele populations for observations made at a single time point. This result calls into question the claim that agreement of data with predictions of the neutral model argues against natural selection as an important factor for the maintenance of genetic variation.

Work on biologically-motivated models for natural selection in a random environment has shown that, under a wide variety of assumptions, two allele models yield diffusion processes with drift and diffusion coefficients of the form

$$\mu(x) = x(1-x)\,[a+b(1/2-x)] \qquad (1)$$
$$\sigma^2(x) = x^2(1-x)^2$$

For symmetric models, which will be the only ones considered here, $a = 0$, and

$$\mu(x) = bx(1-x)(1/2-x) \qquad (2)$$
$$\sigma^2(x) = x^2(1-x)^2$$

For example, for a diploid species with additive alleles in a subdivided environment

$$b = 2/[1-\pi(1-k)] \qquad (3)$$

where π is a measure of the patch structure and k is a measure of the correlation between patches[4]. Biological considerations suggest that $b \geq 2$, with larger b values associated with greater environmental heterogeneity. As another example, if the environments are autocorrelated, we can still obtain a diffusion process for the additive, diploid case, with

$$b = \frac{2}{1+\lambda} \qquad (4)$$

where λ is a measure of the autocorrelation of the encironment[5]. In this instance $1 < b < \infty$, with smaller values of b associated with more positively autocorrelated environments. Other models incorporating dominance and various population structures also give models of the same form as (2), with, in general, $1 < b < \infty$ (ref. 6). The asymptotic density for (2) is a β distribution

$$\Phi_2(x) = \frac{\Gamma(2b-2)}{\Gamma(b-1)^2}[x(1-x)]^{b-2} \qquad (5)$$

which always exists since $b > 1$. Two neutral alleles with equal mutation rates also yield a beta distribution of this form which hints of the reason why random environment models behave, asymptotically, like neutral allele models.

The K-allele extension to (2) may be written, using techniques, similar to those in Gillespie[7], as

$$\begin{aligned} \mu_i(\tilde{X}) &= bx_i(F-x_i) \\ \sigma_i^2(\tilde{X}) &= x_i^2(1+F-2x_i) \qquad i = 1, 2, \ldots, K-1 \qquad (6) \\ \sigma_{ij}(\tilde{X}) &= x_i x_j(F-x_i-x_j) \end{aligned}$$

where $X = (x_1, x_2, \ldots x_{K-1})$, $F = \sum^{K} x_i^2$, and $x_K = 1-x_1-x_2-\ldots x_{K-1}$. We can solve the three allele case by using the zero probability flux condition given in Kimura[8] and following the techniques given in his equations (9.5) to (9.7). If we write the asymptotic density as

$$\Phi_3(x_1, x_2) = \exp(\psi(x_1, x_2)) \qquad (7)$$

we are led to the system

$$\begin{bmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{12} & \sigma_2^2 \end{bmatrix} \begin{bmatrix} \frac{\partial\psi}{\partial x_1} \\ \frac{\partial\psi}{\partial x_2} \end{bmatrix} = \begin{bmatrix} -2\mu_1 - \frac{\partial}{\partial x_1}\sigma_1^2 - \frac{\partial}{\partial x_2}\sigma_{12} \\ -2\mu_2 + \frac{\partial}{\partial x_1}\sigma_{12} + \frac{\partial}{\partial x_2}\sigma_2^2 \end{bmatrix} \qquad (8)$$

which yields, after considerable manipulations

$$\begin{aligned} \frac{\partial\psi}{\partial x_1} &= \frac{2b-5}{3}\left[\frac{1}{x_1} - \frac{1}{1-x_1-x_2}\right] \\ \frac{\partial\psi}{\partial x_2} &= \frac{2b-5}{3}\left[\frac{1}{x_2} - \frac{1}{1-x_1-x_2}\right] \end{aligned} \qquad (9)$$

Integrating this system gives

$$\Phi_3(x_1,x_2) = \frac{\Gamma(2b-2)}{\Gamma((2b-2)/3)^3}[x_1x_2(1-x_1-x_2)]^{(2b-5)/3} \qquad (10)$$

This is a Dirichlet distribution and suggests immediately that the form of the asymptotic density for K alleles will be

$$\Phi_K = C\left[\prod_{i=1}^{K} x_i\right]^{\beta} \quad x_K = 1-x_1-x_2\ldots-x_{K-1} \qquad (11)$$

If we substitute (11) into the equation giving the zero probability flux condition:

$$-\mu_i\Phi_K - \frac{1}{2}\frac{\partial}{\partial x_i}[\sigma_i^2\Phi_K] - \frac{1}{2}\sum\frac{\partial}{\partial x_j}[\sigma_{ij}\Phi_K] = 0 \qquad (12)$$

we discover that the appropriate value of β is

$$\beta = \frac{2(b-1)}{K} - 1 \qquad (13)$$

Thus the asymptotic density for the system (6) is the Dirichlet distribution

$$\Phi_K(X) = \frac{\Gamma(2b-2)}{\Gamma((2b-2)/K^{K})}\left[\prod_{i=1}^{K} x_i\right]^{\frac{2b-2}{K}-1} \qquad (14)$$

This is the same form as the asymptotic density for the K-allele neutral model (see refs 7, 9, 14).

The tractability of the sampling distribution of neutral alleles rests on the assumption that the underlying process has an infinite number of possible allelic states. The natural analogue in our case would be to consider the behaviour of Φ_K as $K \to \infty$. By this procedure we do not mean to imply that there are, in any real population, an infinite number of alleles, rather we view the $K = \infty$ case as an approximation to the behaviour of a large number of alleles. The behaviour of both Φ_K and samples from Φ_K are well known, the most powerful results coming from recent work by Kingman[10].

As $K \to \infty$, the limiting form of Φ_K degenerates, but the distribution of the order statistics

$$x_{(1)} \geq x_{(2)} \geq x_{(3)} \geq \ldots \qquad (15)$$

converges to the Poisson–Dirichlet limit. Kingman[11] has recently proved the remarkable theorem—"a sequence of populations has the Ewens sampling property if and only if it has the Poisson–Dirichlet limit."

Thus the sampling distribution for alleles subjecting to selection in a fluctuating environment according to equation (6) has the same sampling distribution as the neutral allele model. The fact that the Dirichlet distribution yields Ewens' sampling distribution as $K \to \infty$ was apparently shown first by Frank Stewart (unpublished results). Watterson[12] showed independently, that the order statistics of the Dirichlet distribution converge to a limit, and obtained the joint density for the first r order statistics.

For our parameterisation we can write down certain sampling properties immediately using the results of Ewens[3]. The probability that a sample of size $2n$ contains k alleles is

$$Pr[k\,|\,2n] = 1_k\frac{(2b-2)^k}{L(2b-2)} \qquad (16)$$

The probability that a sample of size $2n$ with k allelic types contains n_i alleles of type i is

$$Pr[n_1, n_2, \ldots, n_k\,|\,k, 2n] = \frac{2n!}{k!\,1_k n_1 \ldots . n_k} \qquad (17)$$

where, in (16) and (17)

$$L(y) = y(y+1)(y+2)\dots(y+2n-1)$$
$$= l_1 y + l_2 y^2 + \dots l_{2n} y^{2n}$$

Similarly, we can write down the expected homozygosity as $EF = 1/(2b-1)$, and the frequency spectrum, as used by Yamazaki and Maruyama[13] as $x^{2b-2} + (1-x)^{2b-2}$. Examination of these results shows that heterozygosity increases with *b*. From equations (3) and (4) we are led to the observation that increasing the environmental heterogeneity will increase the heterozygosity, while increasing the autocorrelation will decrease the heterozygosity.

It was quite unexpected that the infinite-allele random-environment model should behave in a manner completely analogous to the neutral-allele model. Ewens's[2] original derivation of the sampling distribution used intuition concerning the behaviour of unique alleles which appear in the population through mutation, continue to segregate for a time, and are then lost. Clearly this line of reasoning does not immediately carry over to the present model because of its inaccessible boundaries. Also, in the neutral model the frequency of a single allele is a Markovian variable whose behaviour can be studied in isolation by lumping the frequencies of all other alleles. This is not the case for the random environment alleles whose individual behaviour depend on *F* and cannot be studied without some knowledge of the actual frequencies of the other alleles.

The assumption of complete symmetry given in equation (6) is probably unnecessary. In the two allele case even asymmetric models give asymptotic β distributions. If this carries over to *K*-alleles, then the results of Kingman[10] indicate that even asymmetric Dirichlet distributions can have the Poisson–Dirichlet limit providing certain conditions are met. The common observation that there are 'too many' rare alleles in many samples from nature[11] can be accounted for by these asymmetries.

The present model is limited in that it does not include finite-population effects. Whether this will seriously affect the behaviour of alleles which are frequent enough to appear in samples is not known.

Finally, since many of the tests for neutrality are based on the asymptotic behaviour of the infinite-allele neutral model, the present results indicate that none of these tests can distinguish neutrality from random-environment behaviour. Included among these tests would be those of Ewens[2] Yamazaki and Maruyama[13], Johnson and Feldman[15] and Nei *et al.*[16].

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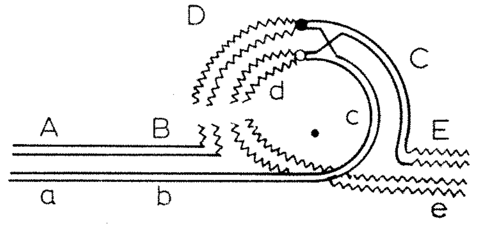
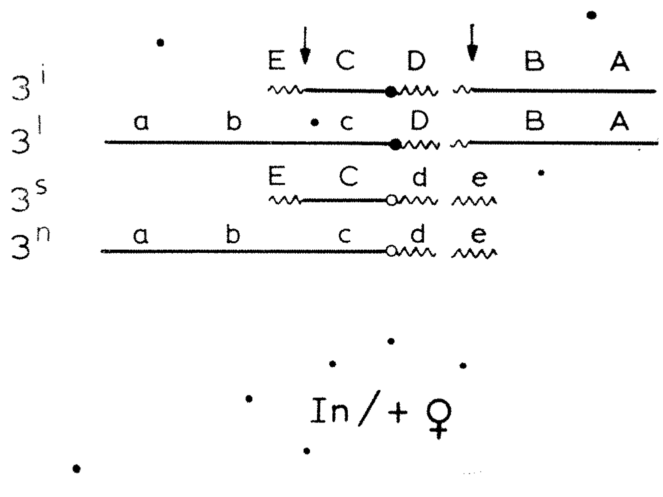
Synthesis of compound chromosomes from a pericentric inversion in the onion fly *Hylemya antiqua*

STRUCTURAL chromosome mutations such as reciprocal translocations[1,2], pericentric inversions[3] and compound chromosomes[4,5] have been considered for the control of insect pests. Their exploitation is based on the capacity to reduce the fertility of a population, and they might be used to introduce a gene into a natural population to render the pest less harmful. This possibility arises from the fact that some structural chromosome mutations confer higher sterility in crosses to wild type, or in the hybrids from such crosses, than in matings within the mutant strain. This property is particularly evident with compound chromosomes which have homologous arms attached to common centromeres rather than to different centromeres. This causes intersterility with wild-type individuals, because all the hybrids carry chromosome duplications and deficiencies. In contrast to studies[5–7] in which compound chromosomes were isolated starting from two almost whole arm reciprocal translocations, I have used a pericentric inversion in the onion fly, *Hylemya antiqua*.

Four different types of chromosomes were produced if crossing over occurred anywhere within the loop of a pericentric inversion heterozygote[8]. Figure 1 shows how these types were discriminated on the basis of total length and arm ratio. The normal (3^n) and inverted (3^i) chromosomes are balanced. The other two crossover chromosomes (3^s and 3^l) both carry duplications and deficiencies and are considered as compound chromosomes, although the breakpoints are not in the immediate vicinity of the centromere. If both sexes were chiasmate, a compound chromosome strain could be made from crosses between inversion heterozygous parents. Because only female onion flies are chiasmate, I looked for males carrying a chromosomal rearrangement which gave products which would be complementary to one of the compound chromosomes produced by the inversion female (Fig. 2). Crosses between inversion females

Fig. 1 Diagram of a pericentric inversion heterozygote and the possible products. 3^i and 3^n are the parental combinations and 3^l and 3^s the results of crossing over in the inversion loop. Arrows indicate breakpoints.





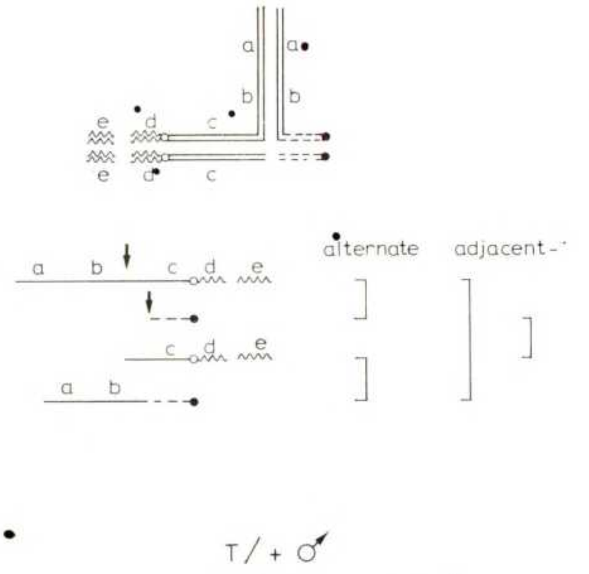


Fig. 2 Diagram of the translocation heterozygote (male) and the two orientation types.

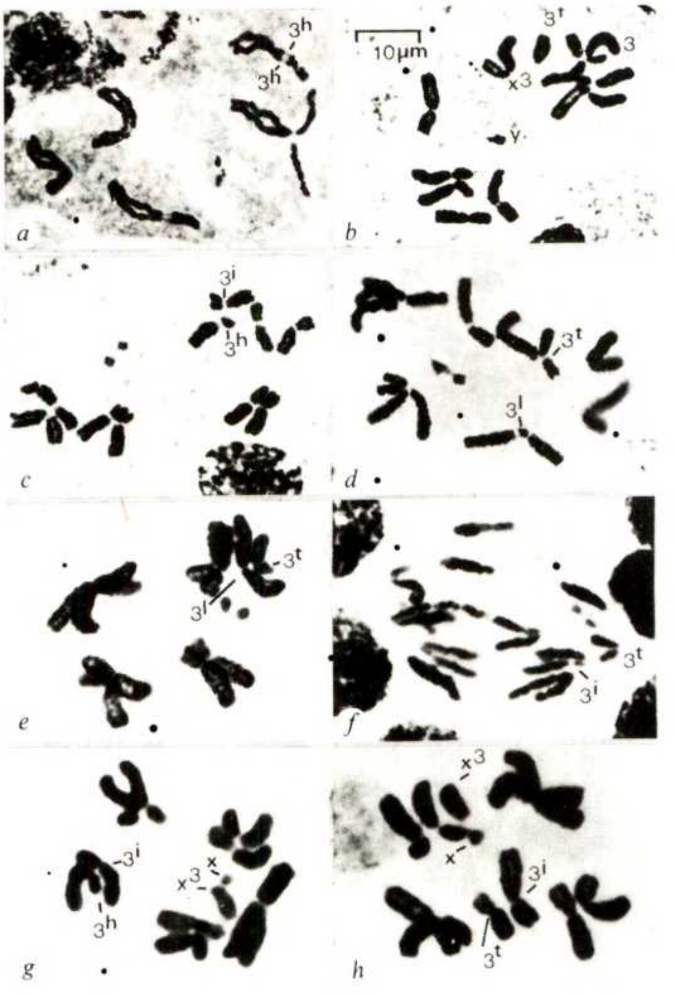


Fig. 3 *a*, Normal karyotype with two 3^n chromosomes, prophase in larva ($2n = 12$). *b*, Translocation heterozygote $3^t3^nX^3Y$ (male), metaphase in larva. *c*, Pericentric inversion heterozygote 3^i3^n, metaphase in larva. *d* and *e*, Larval compound type 3^t3^l, 3^t from T/+ ♂ and complementary 3^l from In/+ ♀. Mitotic metaphase. *f*, Compound type, mitotic anaphase. Note constrictions in 3^l. *g*, $3^i3^nX^3X$, mitotic metaphase in larva. *h*, $3^t3^lX^3X$, mitotic metaphase in larva.

and translocation males produced some progeny with the compound type of chromosome, consisting of a large recombinant chromosome (3^l) from the female and a short complementary translocated chromosome (3^t) from the male. It was intended that subsequent backcrossing of such males with inversion females would give a compound chromosome strain carrying 3^l and 3^s

Cytological analysis (Fig. 3) of an X-linked translocation[9] showed that one of the translocated chromosomes (3^t) resembled the short chromosome (3^s) from the inversion. Four different chromosomes were involved in the translocation, which is a simple one with a breakpoint in the telomeric area of the X chromosome (Figs 2 and 3). Four different types of gametes were produced in equal frequency and 16 different embryonic karyotypes were assumed to occur when the translocation males are crossed to inversion females. As Table 1 shows, five karyotypes were deficient for segment ab (about the distal two-thirds) of the long arm of chromosome 3. Indeed these types were not observed in the larval stage and died as embryos.

Eleven different karyotypes were expected to be viable in the larval stage, but observations on mitotic preparations with somatic pairing revealed only eight types. Of the three missing categories I could not explain the absence of $3^t3^iX^3X$ larvae which are no more duplicated than, for example, the viable 3^i3^nXY type. The $3^i3^nX^3X$ type is tetrasomic for segment ab and therefore probably too duplicated to survive. The $3^s3^nX^3X$ larval karyotype is probably indistinguishable from the translocation heterozygote ($3^t3^nX^3X$). Out of 40 larvae analysed, three were of the male compound type (3^t3^lXY) (Fig. 3*d*, *e* and *f*). Unfortunately no compound karyotype was observed among adult males (30) and therefore no compound strain was isolated. Probably a small terminal deficiency (segment e), distal to the breakpoint in the short arm of chromosome 3 (one-tenth of the chromosome), caused inviability. This was unfortunate because the

Table 1 Gametic combinations after mating of In/+ females and T/+ males

In/+ ♀	T/+ ♂ Alternate 3^tX^3	Alternate 3^nY	Adjacent—1 3^tY	Adjacent—1 3^nX^3
3^n	e d c b a e d c b a	e d c b a e d c b a	e d c Δ e d c b a	e d c bb aa e d c b a
3^i	e d c b a D c Bb Aa	e d c b a D c Bb Aa	e d c * D c Bb Aa	e d c bb aa D c Bb Aa
3^s	e d c b aΔ Ee d C	e d c b aΔ Ee d C	e d c Δ Ee d C	e d c bb aa Ee d C
3^l	e d c b a E D C B A ♀	e d c b a E D C B A ♂	e d c Δ E D C B A ♂	e d c bb aa E D C B A ♀

The letter combinations indicate the presence of duplications and/or deficiencies. The compound type is indicated by an asterisk. The Δ types are expected to die in the embryonic stage. Y is the male determining factor. Photomicrographs of some karyotypes (larvae) are shown in Fig. 3.

presence of a small duplication for a tiny interstitial segment, located between the long arm breakpoints of two rearrangements, usually has little influence[6,9,10].

To avoid the problem of trying to obtain a translocation which is more exactly complementary to the crossover products of the inversion, another suggested method for the production of a compound chromosome strain is the irradiation of inversion heterozygous males to stimulate crossing over in the loop[11]. Then the recombinant chromosomes 3^s and 3^l could be generated through both sexes. The usefulness of the pericentric inversion would be much reduced if the distances of both breakpoints to the centromere are too large. Otherwise crossing over is to be expected between the two compound chromosomes, thus producing normal and inverted chromosomes again and hence intersterility with the wild type would be incomplete. This would prevent use of the system to replace completely a wild population by a harmless form. But in a species with achiasmate males, this sex from such a compound chromosome strain would be fully sterile in matings to wild type. Thus such a strain could be used as a source of material for a sterile male release programme.

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Regulation of dimorphism in the pathogenic fungus *Histoplasma capsulatum*

Histoplasma capsulatum is a dimorphic pathogenic fungus which is the causative agent of the disease histoplasmosis. The disease is worldwide in occurrence and approximately 40 million people have been infected in the USA[1]. In soil the fungus is filamentous, but in the infected host it exists as a budding yeast and it is believed that the phase transition is important in the pathogenicity. In the laboratory, the mycelial form can convert to the yeast phase when the temperature of incubation is shifted from room temperature (25 °C) to body temperature (37 °C). In this report we show that the elevated temperature initiates a series of reactions leading to changes in the intracellular level of 3′,5′-cyclic adenosine monophosphate (cyclic AMP); the shifts in intracellular cyclic AMP levels are an important determinant of the morphological phase of the organism and therefore of its disease producing potential.

Earlier studies[2-9] have already shown that the natural signal of temperature is not by itself critical to cause the phase transition. Rather, temperature seemed to affect the level of sulphydryl groups or the redox potential, which in turn determined the phase of the organism. For example, added sulphydryl, usually in the form of cysteine, could force mycelia into the yeast phase even at 25 °C (ref. 3). Moreover, Rippon showed[10] that a low redox potential imposed even in the absence of added cysteine, stimulated the conversion of mycelia to yeast at 25 °C, whereas yeast converted to mycelia when the medium had a higher redox potential.

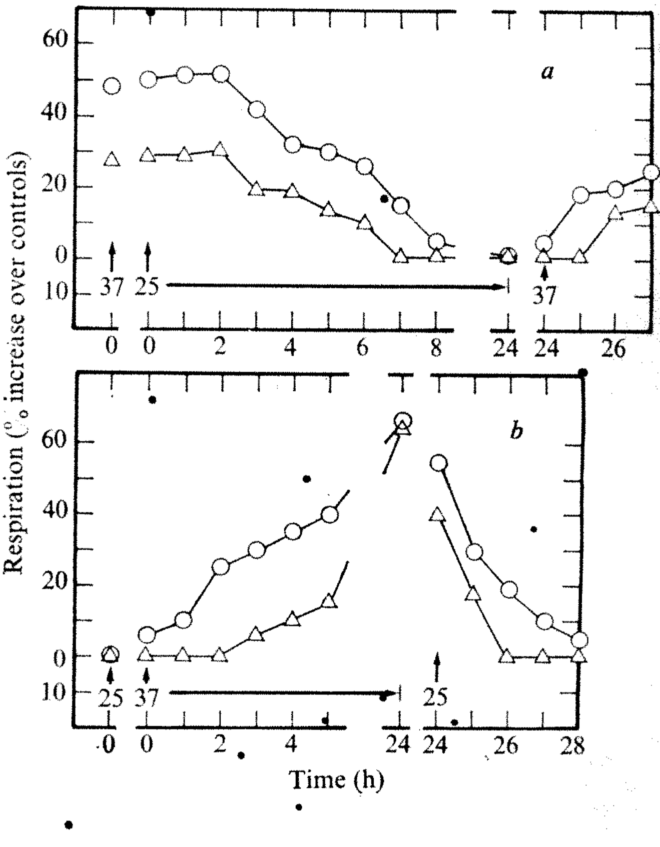
In attempting to analyse further the role of sulphydryl groups, we started from the observations of Garrison that cysteine and cystine were actively transported into yeast by an ATP-dependent permease, and that they stimulated fungal respiration by entering the Krebs cycle as pyruvic acid[11]. Garrison also found that the effects on respiration were a good measure of the active uptake of these compounds.

We found that yeast incubated at 37 °C had a higher level of respiration than mycelia incubated at 25 °C (6.6 and 2.8 nmol O_2 per min per mg dry weight, respectively). When yeast cells were brought to 25 °C, however, respiration immediately fell to the level characteristic of mycelia (2.2 nmol). Similarly, when mycelia were incubated at 37 °C, there was an immediate increase to the level observed with yeast (6.5 nmol). We concluded that the baseline level of respiration in the two phases of *H. capsulatum* was a direct response to temperature of incubation and reflected only the QO_2 of mitochondrial function.

The addition of cysteine or cystine to the medium stimulated the respiration of yeast but not of mycelia. When yeast cells were switched from 37 to 25 °C, respiration fell immediately and the stimulus to respiration of cysteine and cystine gradually diminished to zero over 8 h (Fig. 1). After 24 h of incubation at 25 °C, the temperature was abruptly brought back to 37 °C. Baseline respiration increased immediately and there was a rapid return of the stimulus to respiration by added cysteine (within 5 min) and a later return with added cystine (Fig. 1). When the temperature was switched to 37 °C in the presence of 1 mg ml^{-1} cycloheximide and 1 mg ml^{-1} chloramphenicol, the cysteine-induced change in respiration still occurred. At these concentrations of antibiotics, protein synthesis was less than 20% of control cells[12].

Mycelia incubated at 25 °C did not respond to cysteine or cystine, but when the cells were switched to 37 °C they became responsive to these amino acids within 5 min (Fig. 1). The rapid changes in uptake of cysteine and cystine, even in

Fig. 1 Effect of cysteine (○) and cystine (△). *H. capsulatum* (Down's strain mating type [−]) was grown in 2% glucose 1% yeast extract (GYE) in a New Brunswick rotary shaker. An inoculum of 10 mg dry weight of mycelia per ml was incubated at 25 °C, and an inoculum of 5 × 10^6 yeast cells per ml was incubated at 37 °C. Oxygen consumption was measured using a Clark electrode. The dry weights were determined by filtering 5 ml culture through 0.22-μm Millipore filters and drying the filters at 60 °C to constant weight. Daily fresh solutions of cysteine (pH 6.4) and cystine were prepared. The final concentrations were respectively 10 mM and 21 μM. Rate of growth of the two phases as determined by dry weights was the same. *a*, Yeast cells; *b*, mycelial cells.



cycloheximide–chloramphenicol-treated cells, suggested that this process did not require new protein synthesis and that transport protein(s) may be present on cell membranes of both phases of *H. capsulatum*, but only active at 37 °C.

If the action of temperature is to change membrane redox potential, and this can be facilitated by activating a permease for cysteine uptake, fungal dimorphism may be controlled by some critical sulphydryl groups, or by a general change in reducing state. To test this, *p*-chloromercurphenisulphonic acid (PCMS), a sulphydryl-blocking agent, and dithiothreitol (DTT), a sulphydryl protector, were tested for their effects on the phase transition. Mycelia were exposed to 100 μM PCMS for different lengths of time (5 min to 2 weeks) and were then washed twice, resuspended in fresh medium without PCMS and plated on GYE agar plates at 37 °C. The cells grew continuously after this treatment but never reverted to the yeast form (Fig. 2). PCMS (100 μM) also accelerated the transition of yeast to mycelia at 25 °C. After exposure to PCMS and incubation at 25 °C, yeast transformed to mycelia in 5–6 d instead of the usual 12–14 d. Even yeast incubated at 37 °C began to transform to mycelia in the presence of PCMS, but reverted to normal yeast when PCMS was washed out.

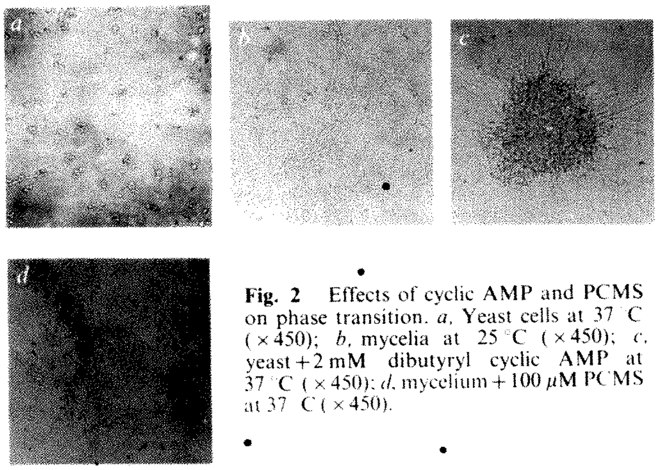


Fig. 2 Effects of cyclic AMP and PCMS on phase transition. *a*, Yeast cells at 37 °C (×450); *b*, mycelia at 25 °C (×450); *c*, yeast + 2 mM dibutyryl cyclic AMP at 37 °C (×450); *d*, mycelium + 100 μM PCMS at 37 °C (×450).

Opposite effects were found when cells were treated with DTT. For example, when yeast were incubated at 25 °C in the presence of 5 mM DTT, transformation to mycelia did not occur (although in this case the transition to mycelia still occurred when DTT was removed from the medium). Neither PCMS nor DTT were toxic to either phase of *H. capsulatum* at the concentrations used.

The effects of PCMS and DTT argue that sulphydryl groups, most likely at the surface of the cell, are critical for determining the phase of *H. capsulatum*. The irreversibility of the PCMS effect was unexpected, but also suggested that some surface feature was critical. The next question was how critical sulphydryl groups, especially at the membrane of the organism, could regulate a complex transition of yeast to mycelium. One possible explanation was that this phenomenon was similar to the effects of many hormones on eukaryotic cells, which often begin with a membrane change that modifies the intracellular level of a cyclic nucleotide.

In *H. capsulatum*, we found that the level of cyclic AMP was about five times higher in the mycelial phase than in the yeast phase. Cyclic AMP was assayed in yeast and mycelia by the radioimmunoassay of Steiner *et al.*[13] or with the Amersham–Searle cyclic AMP assay kit. The cells were collected by filtration, washed three times in ice-cold water, suspended in either 5% TCA, 0.1 N HCl or 0.05 M Tris-HCl buffer, *p*H 7.5, containing 1 mM 3-isobutyl-1-methylxanthine (Aldrich Chemicals). Glass beads (diameter 0.45–0.50 mm) were added to the suspension and the cells were disrupted at 4,000 r.p.m. for 3 min in a B. Braun Cell Homogeniser MSK, cooled with liquid CO_2. The resulting homogenates were centrifuged and an aliquot of the supernatant was lyophilised directly, or, in the case of cells homogenised in TCA, after extraction in ether. The dried material was resuspended in the appropriate buffer, and the cyclic AMP content determined. The recovery of added cyclic AMP in controls was 80–90%. The supernatant of cells homogenised in Tris buffer containing methylxanthine and precipitates of HCl and TCA-extracted cells redissolved in 1 N NaOH were assayed for protein according to Lowry *et al.*[14]. The average of three determinations showed that mycelia contained 46.9 and yeast 9.8 pmol cyclic AMP per mg protein. In addition, 2 mM dibutyryl cyclic AMP or 5 mM theophylline added to yeast cultures resulted in the conversion of yeast to mycelia, even at 37 °C, the temperature characteristic for the yeast phase (Fig. 2).

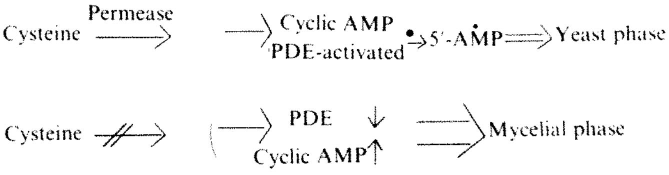


Fig. 3 Working hypothesis for role of cysteine and cystine in morphogenesis of *H. capsulatum*.

Thus, although the role of cysteine and cystine in morphogenesis of *H. capsulatum* is not understood, it seems likely that they satisfy a requirement for critical sulphydryl groups that then determine the level of intracellular cyclic AMP. We do not know how this might occur, but possibly, as in *Escherichia coli*, the activity of cyclic AMP-phosphodiesterase (cyclic AMP-PDE) requires the presence of a reducing substance[15]. The redox potential, governed by sulphydryl groups, could then have a regulatory role in the level of cyclic AMP, which in turn would control morphogenesis. Therefore, we suggest as a working hypothesis the schema of Fig. 3. At 37 °C the cells are able to bring cysteine to critical membrane sites. There it would activate cyclic AMP–PDE, thereby lowering intracellular cyclic AMP and maintaining the yeast phase. When the temperature is switched to 25 °C, transport of cysteine decreases and cyclic AMP–PDE activity falls. This results in an increase in cyclic AMP levels and a shift to the mycelial phase.

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The role of chemotaxis in the ecology of bacterial pathogens of mucosal surfaces

ALTHOUGH the ecological role of bacterial chemotaxis has been the object of considerable speculation, little is known of its role in nature[1]. Moreover, chemotaxis as a factor in the interaction between the mammalian host and its indigenous or pathogenic microflora remains unexplored. Studies with *Vibrio cholerae* and other intestinal pathogens have shown that bacterial association with the mucosa is influenced by bacterial motility, adhesion to brush border membranes, the presence of an indigenous flora and by the inhibitory effects of local antibodies

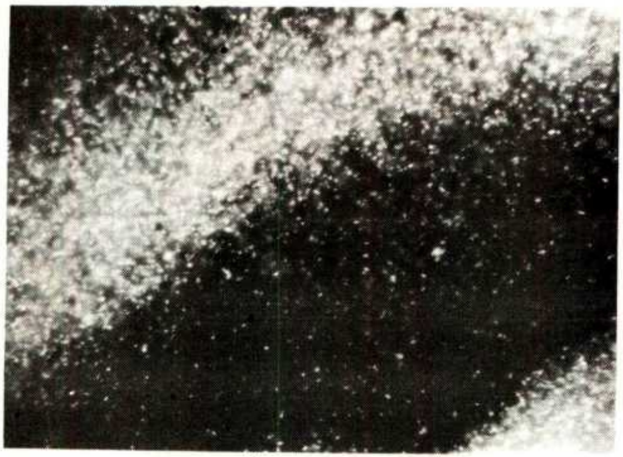

Fig. 1 A chemotactic strain of *S. typhimurium* forming a receding band at some distance from the mucosal surface. The bright triangular area in the lower right corner is the surface of the mucus gel overlaying the villi. The band had originally formed within the mucus gel and some bacteria have been retained there. The diffuse light within the band emanates from bacteria outside the focal plane of the microscope. The diffuse light in the mucus is due to light scattering by the gel itself. Darkfield (×110).

(summarised in refs 2, 3). We have shown previously that a pepsin digest of rabbit intestinal mucosa (PMS) inhibits the association of cholera vibrios and *Salmonella enteritidis* with rabbit intestine, and speculated that this extract was inhibitory because it neutralised the adhesion of the bacteria to a hypothetical receptor in the mucus gel[3]. We report here that PMS neutralises a positive chemotactic response of several bacterial species to the mucosa and in that way reduces bacterial association with intestinal tissue. Chemotaxis thus seems to be one of several mechanisms controlling interactions of bacteria with mucosal surfaces.

Observation under a phase-contrast or darkfield microscope of pieces of mouse or rabbit small intestine immersed in a suspension of *V. cholerae* revealed that very high concentrations of vibrios aggregated at the villous surface of this tissue in approximately 1 min. Three non-chemotactic vibrio mutants were selected using a modification of the method of Aswad and Koshland[4] and differed from the parent strain in that they did not accumulate at the surface of intestinal tissues, but remained randomly distributed throughout the suspending buffer. Similarly rapid attraction to mucosal tissue was shown by *Escherichia coli* (AW 405) and *Salmonella typhimurium* (SL 1634), but not by their non-chemotactic mutants (cheB 590 and SL 2538, respectively). None of the mutants used in this study differed from their respective parents in the rate of motility as determined by direct microscopic observation.

After chemotactic strains of the three bacterial species had accumulated on the surface of intestinal tissues for a period of approximately 2–5 min, the bacterial accumulation began to move away to form a clearly visible band of motile bacteria some distance from the tissue surface (Fig. 1). The distance of separation increased with time until the band eventually reached the edge of the coverslip. No such bands were formed by any of the non-chemotactic mutants. The formation of these bands may be caused by the local consumption of oxygen at the tissue surface or by the generation of metabolites with negative chemotactic activity by the tissue and/or by the bacteria. Such negative chemotactic gradients would compete with the positive chemotactic gradient generated by the tissue. Accordingly, the bacteria would be expected to accumulate in an area defined by the interaction of opposing gradients. Similar banding phenomena in broth or agar cultures of various bacteria have been described by others[5-7]. Whatever the actual mechanisms may be, our direct microscopic observations indicate that strikingly effective chemotactic attraction and repulsion of a variety of bacteria can occur at the mucosal surface.

Agar blocks infused with an ultrafiltrate of PMS could be substituted for the mucosal tissue slices. Such blocks attracted *E. coli*, vibrios and salmonellae to their surfaces, indicating that PMS contained one or several attractants. Agar blocks soaked in buffer had no effect. Moreover, the addition of PMS to the bacterial suspension reduced or prevented the chemotactic attraction of vibrios to rabbit mucosal tissue in the microscopic assay. By analogy with other chemotactic systems[7] it seems likely that the attractants in PMS saturated those chemotactic receptors of bacteria which normally reacted with the attractants emanating from the mucosa, thereby blocking the reactivity of the bacteria. These interactions were also demonstrated with Adler's capillary test for chemotactic activity[8]. Cholera vibrios were attracted into capillaries containing dilutions either of PMS or of a soluble fraction obtained by centrifuging freshly prepared scrapings from the mucosa of rabbit small intestine (Muc. Sn.) (Table 1). The latter preparation presumably contained the chemotactic mediators of mucosal tissue in relatively unaltered form. When PMS was added to the bacterial suspension as well as to the capillaries, no chemotactic gradient could form and no migration into the capillaries occurred. Similarly, the presence of PMS in the vibrio suspension considerably reduced the entry of vibrios into capillaries containing Muc. Sn. (Table 1), suggesting that at least some of the attractants in PMS and Muc. Sn. were similar. No response in the capillary test was observed with the non-chemotactic mutant vibrios.

The effect of chemotaxis on the association of vibrios with intact slices of rabbit ileal tissue was determined as described before[3], with the modification that the tissues were incubated in buffer containing 5×10^5 cells per ml of each of the chemotactic parent strain plus one non-chemotactic mutant. The proportions of mutant to parent strains that remained on the washed tissues were determined by preparing agar plate cultures and subsequently transferring a number of individual colonies to semisolid tryptone agar[8]. The proportion of non-chemotactic mutants associated with the tissue slices was 3.0 to 3.8 times lower than their proportion in the suspending fluid, indicating that the mutants had a significantly lower chance of associating with the mucosal tissue than the parent strain (Table 2). PMS in the suspending medium had no effect on the association of the non-chemotactic mutants with tissue slices. In contrast, PMS in the suspending medium reduced the association of the parent strain with mucosal slices by a

Table 1 Chemotactic attraction of *Vibrio cholerae* into capillary tubes and its inhibition by a preparation derived from intestinal mucosa

Content of capillary	Buffer	50% PMS*	5% PMS*	50% PMS*	Muc. Sn.† 2%	Muc. Sn.† 2%	Muc. Sn.† 2%
Content of bacterial suspension	Buffer	Buffer	Buffer	50% PMS*	Buffer	50% PMS*	5% PMS*
No. of bacteria in capillary‡ ($\times 10^{-4}$)	3.5§ 7.0§	142 97	51 51	4.0 7.0	40 34	8.5 14	9.5 12

* Ultrafiltrate of pepsin digested rabbit mucosal scrapings.

† Supernate of centrifuged, freshly prepared rabbit mucosal scrapings.

‡ Capillaries of 0.27 mm diameter, filled with 5 µl Tris buffer (*p*H 7.4) containing the materials indicated, were dipped for 60 min into vibrio suspensions. The number of vibrios which had invaded the previously sterile capillaries were then counted in a Petroff–Hausser chamber.

§ Duplicate determinations.

Table 2 Reduced invasion of mucosal tissue by non-chemotactic mutants of *Vibrio cholerae*

Parent strain plus mutant no.*	Fraction of non-chemotactic vibrios in suspending fluid*	Fraction of non-chemotactic vibrios on washed mucosa†	Factor: fluid/mucosa
8	41/93 (44.1%)	23/155 (14.8%)	3.0
6	39/73 (53.4%)	22/155 (14.2%)	3.8
31	61/137 (44.5%)	23/155 (14.8%)	3.0

* Slices of rabbit mucosal tissue were incubated without shaking for 8 min in a suspension of vibrios containing approximately equal concentrations of the chemotactic parent strain plus one non-chemotactic mutant as indicated.
† Based on counts of 31 colonies each cultured from 5 mucosal tissue slices.

factor of two to four[3]. Consequently, when the chemotactic attraction of the parent strain towards the mucosa was blocked by PMS, its ability to associate with the mucosa was reduced to a level similar to that of the non-chemotactic mutants.

Results obtained in three different experimental systems thus indicate that chemotactic mechanisms can promote as well as inhibit the initial invasion of mucosal surfaces by bacteria. Attractive and possibly repellent substances produced by the tissue are obviously important factors in these processes, but chemotactically active substances such as PMS in the medium bathing the mucosal surface seem to be equally potent modifiers of bacterial invasion. It is tempting to speculate about the *in vivo* role of chemotaxis in the interaction between bacteria and their mammalian host. For example, in the initial stages of infection an intestinal pathogen is rapidly propelled along the small intestine in a bolus of digesta, and the duration of its contact with any given area of the mucosal surface may not be longer than a few seconds. The ability of chemotactically attracted bacteria to quickly approach and penetrate the mucus gel may well be of even greater importance in that situation than in the invasion of the statically incubated tissues studied in the present experiments. Indeed, Chet and Mitchell[1] quote data showing rapid chemotactic movement of bacteria across and against fluid flow planes in flowing streams, that is, in conditions which kinetically resemble those of the small intestine. Furthermore, intestinal contents almost certainly contain chemotactically active substances which may either promote or inhibit chemotactic attraction of bacteria into the mucosa. Such a phenomenon may relate to certain aspects of the long-recognised relationship between diet and susceptibility to infection, a phenomenon for which exact mechanisms are still to be delineated[9]. It is also possible that chemotactically active substances added to the diet may exert a prophylactic effect against infections such as traveller's diarrhoea. The association of indigenous bacterial flora with the mucus layer of the large intestine may also be governed in part by chemotactic mechanisms.

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Helical shape and wall synthesis in a bacterium

THE main skeletal components of most bacterial cell walls are complex polymers called peptidoglycans[1]. The primary chemical structures of these and other wall components are well understood but little is known about the arrangement of the molecules in the wall or how new wall material is incorporated during growth[1]. We report here that several factors cause cells of *Bacillus subtilis*, normally straight rods (Fig. 1*b–e*), to assume a regular helical shape. We suggest a model for the structure and synthesis of Gram positive cell walls which could explain these observations. Explanations suggested by Mendelson[2] for an apparently different type of helical growth in *B. subtilis* do not account for our results.

In the model which most satisfactorily explained his observations, Mendelson[2] suggested that the pattern of wall growth in his mutant, and perhaps other strains, results in the rotation of the two ends of a cell relative to one another. When, in his strain, both ends of a cell filament remain attached to the old spore case this rotation is prevented and the resulting torque is relieved by the whole filament distorting to a helix.

We have observed that helices predominate among the abnormally-shaped cell filaments that occur in cultures of *B. subtilis* in the following conditions (f = approximate frequency of helical filaments in the populations).

(1) In medium supplemented with the local anaesthetics chlorpromazine (3×10^{-5} to 4×10^{-5} M; 37 °C) or methochlorpromazine (5×10^{-5} to 6×10^{-5} M; 37 °C); $f = 50\%$.

(2) In medium containing penicillin G (0.1–0.25 U ml^{-1}; 30 °C); $f = 1\%$ (Fig. 1*b–e*).

(3) Certain mutants of strain 168 (ref. 3) resistant to the detergent Triton X-100 (unpublished work) grow predominantly in a helical form, especially near the end of exponential growth. $f > 95\%$ (Fig. 1*a* and *f*).

(4) In cultures restarting growth after subculturing from stationary phase. $f < 1\%$.

(For (1) and (2) exponentially growing cells were subcultured, to an initial density of 2×10^6 ml^{-1}, into media containing the drugs. Strain 168*ind*⁻ (ref. 3) and Penassy broth (Difco) were used in (2), (3) and (4) but wild-type Marburg strain and nutrient broth (Difco) in (1).)

Our bacteria were not grown from spores, did not appear as closed loops (Fig. 1) and were grown in liquid media. Therefore, unlike Mendelson's example, anchorage of cell ends could not have contributed to their morphogenesis. In addition, the helices we observed were usually much more tightly coiled (Fig. 1).

Mendelson considered it improbable that helical shape could result from unequal growth rates at different points around the cell circumference because of the complexity of the necessary growth pattern[2]. This argument also applies to our observations—perhaps even more so because of the variety of circumstances involved.

Highton and Hobbs[4] described penicillin-induced helical growth, very similar to that reported here, in a penicillin-resistant mutant of *B. licheniformis*. They found abnormal wall thickening at the inside curvatures of bent cells. Similar thicken-

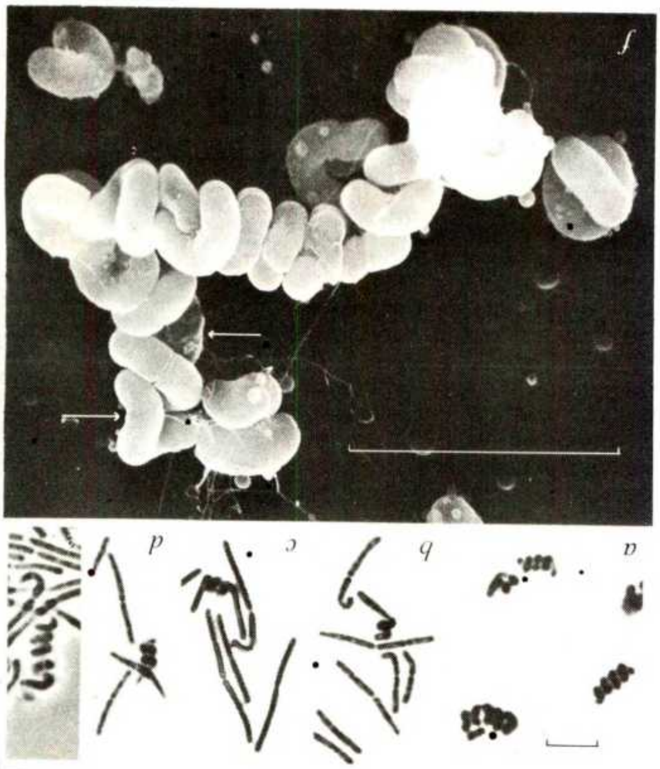


Fig. 1 *a* and *f*, Triton-resistant mutant (TR61) grown in Penassy broth. Arrows in *f* show points of change in direction of helix; *b–e*, normal (straight) and helical forms of strain 168*ind*⁻ grown in Penassy broth containing Penicillin G (0.25 U ml^{-1}); *a–e*, light micrographs of cells fixed in 1% formalin and dried on to slides; *a–d*, stained with crystal violet; *e*, unstained, and phase contrast illumination; *f*, critical point dried bacteria in the scanning electron microscope. Bar, 5 μm.

ing was present in an irregularly bent mutant of *B. subtilis*[5]. The thickening was attributed to an altered and irregular growth pattern[1] or to lack of wall expansion due to insufficient lytic enzyme[5]. Without an explanation for the development of a regular pattern of wall thickening, these suggestions alone cannot account for long regular helix formation.

Helical shape may also occur if the cell wall consisted, at least in part, of fibres wound in a helical manner around the cell. If the quantity or quality of right- and left-hand wound fibres were unequal, and if the fibres were in a state of tension or compression, the cell might assume a helical shape, the direction of which would depend on which class of fibre predominated. This characteristic has been mentioned in a theoretical analysis of the structure of body walls of certain worms[6].

Helical shape in a bacterium may occur in the following circumstances.

(1) If, in a cell wall normally composed of unequal quantities or qualities of right- and left-hand wound fibres, tension or compression forces along the fibres were introduced through imbalance in wall formation, expansion and turnover.

(1*a*) As (1) but fibres are normally wound in only one direction in the same wall area or at the same time. Helical cell shape may then also result from the inclusion of abnormal contrarily wound fibres.

(2) If, in a cell wall normally containing equal quantities and qualities of right and left hand wound fibres under tension or compression, the equality is disturbed.

(3) If, in a cell wall where the fibres are not normally helically arranged (that is, are parallel or at right angles to the cell's long axis), the pattern is disturbed to give unequal right- and left-hand wound helical components.

None of these models involves anchorage of cell ends and therefore they can explain our observations.

There is evidence that Gram positive cell wall material is synthesised in the form of fibres. During early stages of reversion of *B. licheniformis* protoplasts to bacilli, murein and teichoic acid are in the form of distinct fibres[7]. In addition, a fibrillar structure has been seen in sections[1] although the latter observation is not a reliable guide to wall structure. Pooley[8] suggested that wall material in *B. subtilis* is arranged in somewhat discrete fibres or sheets to facilitate the complex expansion and reorganisation that accompanies growth.

The glycan chains of *B. subtilis* peptidoglycan are too long to be oriented radially in the wall[9] but are short (< 200 nm (ref. 9)) compared with the cell circumference (~ 1.8 μm, unpublished data) and fibres[7]. It is, however, easily conceivable that fibres consist of several overlapping cross-linked glycan chains. They could be formed in a manner comparable with the apparent mode of cellulose fibre synthesis in higher plants[10], that is, by membrane-embedded enzyme complexes. Whole fibres could be cross-linked after deployment in the wall.

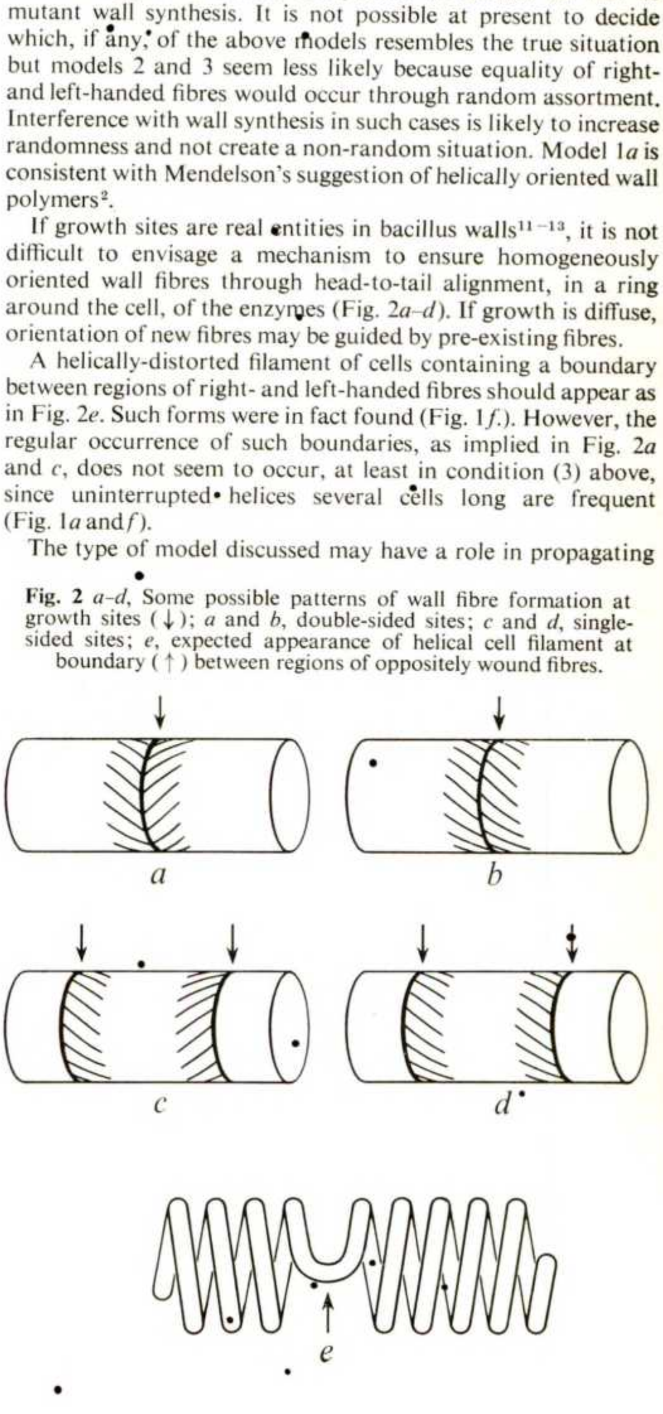
The ease with which wall metabolism can be unbalanced is not surprising considering its biochemical and spatial complexity. The fact that helical cell shapes have been observed by ourselves, Mendelson[2] and Highton and Hobbs[4] in a variety of circumstances suggests a helical component in normal as well as mutant wall synthesis. It is not possible at present to decide which, if any, of the above models resembles the true situation but models 2 and 3 seem less likely because equality of right- and left-handed fibres would occur through random assortment. Interference with wall synthesis in such cases is likely to increase randomness and not create a non-random situation. Model 1*a* is consistent with Mendelson's suggestion of helically oriented wall polymers[2].

If growth sites are real entities in bacillus walls[11–13], it is not difficult to envisage a mechanism to ensure homogeneously oriented wall fibres through head-to-tail alignment, in a ring around the cell, of the enzymes (Fig. 2*a–d*). If growth is diffuse, orientation of new fibres may be guided by pre-existing fibres.

A helically-distorted filament of cells containing a boundary between regions of right- and left-handed fibres should appear as in Fig. 2*e*. Such forms were in fact found (Fig. 1*f*.). However, the regular occurrence of such boundaries, as implied in Fig. 2*a* and *c*, does not seem to occur, at least in condition (3) above, since uninterrupted helices several cells long are frequent (Fig. 1*a* and *f*).

The type of model discussed may have a role in propagating

Fig. 2 *a–d*, Some possible patterns of wall fibre formation at growth sites (↓); *a* and *b*, double-sided sites; *c* and *d*, single-sided sites; *e*, expected appearance of helical cell filament at boundary (↑) between regions of oppositely wound fibres.



the normal very regular shape of *B. subtilis* and could readily explain, in principle, the normal helical shape of spirilla[14].

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Neoplastic transformation of hamster embryo cells irradiated *in utero* and assayed *in vitro*

INDUCTION of neoplastic transformation *in vitro* by X rays[1-5] and neutrons[4] has been reported. Using short term cultures of hamster embryo cells we found previously[1-4] that transformation by X rays can be detected with doses as low as 1 rad. The rate of transformation increases with dose reaching a peak of 1% between 150 and 300 rad. This frequency of neoplastic transformation *in vitro* is much higher than the frequency of radiation-induced tumours observed after exposing animals to similar doses of radiation[6]. We report here that malignant transformed cells can be obtained from embryos irradiated *in utero* and assayed *in vitro* and that the frequency of transformation is at least tenfold lower than that obtained when the irradiations are performed *in vitro*, and thus closer to the incidence in animals.

Pregnant Syrian hamster strain LSH (Lakeview) were irradiated at 12 d of gestation with 300 rad of X rays. The embryos were surgically removed within 30 min after irradiation. Passaging was carried out as described below and in Table 1. We tried to conserve in culture as many of the original cells as was technically possible. The cultures were passaged twice a week and each flask was handled individually. At every passage half the population of the freshly dissociated cells, resuspended in fresh medium, was replated back into the original flask while the rest of the cells were seeded into a new flask containing 5×10^4 feeder cells (syngeneic cells irradiated with 4,000 rad (ref. 7)). After the third passage cells were replated only into the flasks from which they had been removed. Embryos at 12 d of gestation from non-irradiated hamsters of the same litter served as controls and were cultured as above.

Transformed cells were initially identified by their altered morphology and growth patterns[1-4]. An additional criterion of transformation is the ability of cells to grow in low serum concentration, which will not support the growth of normal cells. Cells which, from morphological considerations, seemed to be transformed were isolated and grown into mass cultures in medium fortified with either 10% or 1% foetal calf serum. The ability of the cells to proliferate at the lower serum concentration served as an immediate and simple check of their transformed nature.

Within 30–40 d (7–9 passages) after primary cultures had been established from irradiated embryos, cell transformation was observed in four out of five experiments. In each experiment five to six foci were isolated and a number of cell lines were established. Two established transformed lines designated LSH 1 and LSH 5, each from a different experiment, have been studied extensively. The results of these investigations are summarised for LSH 5 in Table 1.

Chromosome analysis carried out at early passages after transformation (see Table 1) indicated that the modal number of the transformed cells was close to the diploid, 44. The number of chromosomes per cell in LSH 1 ranged from 40 to 56; modal chromosome number 46 (in 40% of the cells), and in LSH 5 the range was 38–60, modal chromosome number 48 (in 30% of the cells). There was no difference between the banding patterns of specific metaphase chromosomes from the transformed cells as compared with the normal.

While the two lines differed from one another in their saturation density, they both reached a higher saturation density than the normal cells in the two sets of conditions. The doubling time of the transformed cells of both cell lines was about 16 h which does not differ greatly from that of normal cells grown in optimal condition (Table 1).

A scanning electron microscopy study indicated that the cells which express transformation *in vitro* following X-ray irradiation *in utero* are similar to those transformed *in vitro*[3].

Changes in cellular surface microstructure, following neoplastic transformation, result in the ability of the transformed cells to be agglutinated by low concentrations of plant lectins[8-10]. The normal counterparts are not agglutinable at similar concentrations (Table 1). The ability of transformed cells to grow in agar is associated with their loss of anchorage dependence. Normal cells derived from solid tissue do not form colonies in semi-solid agar. Following an incubation period of 2 weeks in 0.33% agar[11] the control plates showed no sign of colonies, while the plates containing the transformed cells exhibited distinct colonies with a plating efficiency of 5% and 10% at passage nine for LSH 1 and 5, respectively.

We investigated some biochemical markers associated with the neoplastic state of the cells; namely the fibrinolytic activity

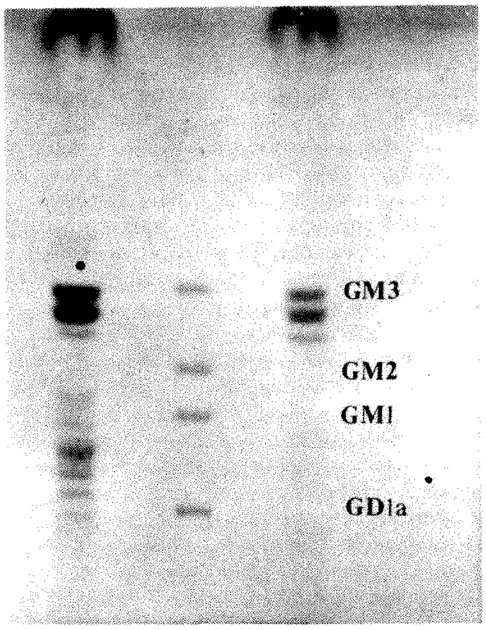

Fig. 1 Thin-layer chromatogram of gangliosides from normal hamster embryo cells and X-ray transformed LSH 5 cells. Lane 1 gangliosides in control cells, lane 2 ganglioside standards, and lane 3 gangliosides in X-ray transformed cells. Note that in the transformed cells gangliosides higher than GM3 are absent. Analysis was by Drs P. H. Fishman, R. O. Brady and R. M. Bradley, using published procedures[17].

Table 1 Characteristics of LSH 5 cells transformed *in utero* by X-irradiation

	Normal (Secondary culture)	Transformed (4th to 30th passage after isolation)	Tumour (1st to 10th passage) after reculturing
Karyotype	Diploid	Near diploid	Aneuploid
Growth in culture	Monolayers	Multilayers	Multilayers
Saturation density no. of cells cm^{-2}			
in 10% serum	7.6×10^4	1.8×10^5	1.5×10^5
in 1% serum	Minimum growth	10^5	10^5
Doubling time			
in 10% serum	16±2 h	14±2 h	16±2 h
in 1% serum	90±5 h	24±2 h	25±2 h
Morphology—scanning electron microscope	Flat and regular surface features except during mitosis	Pleomorphic with complex surface features throughout the cell cycle	Pleomorphic with complex surface features throughout the cell cycle
Agglutinability by con A (20 μg ml^{-1}) and WGA (50 μg ml^{-1})	−	+	+
Plating efficiency			
in 10% serum	2.75±1%	52.5±5.5%	49.5±6.5%
in 1% serum	0.05±0.05%	5.5±2.5%	6.0±2.5%
Efficiency in 0.33% agar	0	10%	30%
Plasminogen activator	low	high	high
Lysosomal hydrolases: phosphatase	low	high	high
Production of MIF	absent	high	high
Gangliosides	Presence of full range of gangliosides	Absence of gangliosides higher than GM$_3$	Absence of gangliosides higher than GM$_3$
Presence of radiation leukemia virus	—	—	—
Tumorigenicity (invasive malignant sarcomas and carcinomas)	none	29/30	Transplantable 12/12

Cells were cultured from a malignant sarcoma which grew in the syngeneic host following subcutaneous (s.c.) injection of 10^5 of the transformed cells and from normal embryonic cells of a secondary culture. Irradiations (see text) were performed in a parallel opposed set-up, so that animals were irradiated simultaneously from above and below with two X-ray tubes operated at 180 kV, 30 mA, with added filters of 0.28 mm aluminium and 0.28 mm copper. The dose rate in the position occupied by the animals was 250 rad min^{-1}. Embryos were removed within 30 min of irradiation. Primary cultures were established by progressive dissociation of the minced fresh tissue[11]. Cells (2×10^7) were seeded into eight 75 cm^2 flasks (Falcon) and incubated at 37 C in a humidified incubator with a constant flow of 5% CO$_2$ in air. Medium, composed of Dulbecco's modified Eagle's medium fortified with 10% foetal calf serum (Grand Island Biological), was replaced 12 h after seeding and the cells were allowed to grow to confluency. Cells were passaged twice a week at a ratio of 1:2 (see text). Transformed foci were observed at passages 7–9 in 6 or 10 of 64 flasks. Foci were isolated using reported techniques[7]. Chromosomes were analysed at the 6th passage after isolation using quinacrine fluorescent banding[21]. Saturation densities and doubling time were determined by seeding 10^5 cells in 60-mm Petri dishes in medium containing 1% or 10% foetal calf serum and following cell growth daily with a Coulter counter. Methods used for scanning electron microscopy[3], testing for concanavalin A (con A) 20–50 μg ml^{-1} and wheat germ agglutinin (WGA) 20–50 μg ml^{-1} induced agglutination[10], colony formation in 0.33% agar[11], testing plating efficiency (PE; PE = (No. clones countered/no. cells plated) × 100), and assays of fibrinolytic activity (plasminogen activator)[12], migration inhibitory factor (MIF)[15], lysosomal acid hydrolases[16], ganglioside content[17,18] and presence of radiation leukaemia virus[19] were as reported before. Tumorogenicity was established by s.c. injection of 10^5–10^7 cells in 0.1 ml of serum-free medium into 2-week-old syngeneic hosts or into the cheek pouch of allogeneic LHC strain hamsters. Portions of the tumours were fixed and stained for histopathological examination and other portions were implanted s.c. in 2-week-old syngeneic hosts; a third portion of the tumours was cultured *in vitro*[11]. Cultured tumour cells were studied as described for the transformed cells. In quantitative studies cells were freshly dissociated from embryos irradiated with 300 rad *in utero* and cloned on feeder cells[1,2]. Seeding levels were 2×10^3–10^4 cells per 60-mm Petri dish (Falcon); 400 dishes were used in each assay. Non-irradiated embryos cultured in the same manner served as controls. The criteria for transformation and the analysis of transformation rate were as before[1–4].

of the transformed cells, the release of macrophage inhibitory factor (MIF), the pattern of cellular lysosomal hydrolases and the ganglioside content of the cells. Fibrinolytic activity[12,13] of the transformed cells was over 20-fold greater than normal; MIF activity[14,15] was confined exclusively to the transformed cell population. Out of 15 cellular acid hydrolases which were determined fluorometrically[16], only acid phosphatase seemed to be significantly elevated in the transformed cells (a twofold increase over the normal). While the full range of gangliosides[17,18] was present in the normal cells the transformed cells showed a complete absence of gangliosides larger than GM$_3$ (see Fig. 1).

Experiments performed by Drs H. S. Kaplan and A. Dèclève, using a reported immunofluorescent assay[19], demonstrated that there was no radiation leukaemia virus (Rad LV) present in the radiation transformed cell lines.

The critical assay for the malignant nature of transformed cells is their ability to give rise to tumours when injected into appropriate hosts. The cells transformed *in utero* by radiation and cultured into cell lines *in vitro* have all given rise to tumours in syngeneic LSH hamsters and also in hamsters of the LHC strain. All animals were 2 weeks old when injected. The tumours were characterised as non-metastesising malignant invasive sarcomas or carcinomas (Fig. 2). They grew progressively and killed the animals.

The tumours are transplantable and have been cultured *in vitro*. The morphological and biochemical properties of the cells cultured from the tumours are similar to those of the original transformed cells (Table 1).

Malignancy varied from one transformed cell line to another (details to be reported elsewhere). In the 2 lines discussed above 10^5 cells of LSH 5 and 10^6 cells of LSH 1 can induce a palpable tumour, 2 cm in diameter, within 2 weeks after injection. Our quantitative studies on transformation *in utero* are outlined in the legend to Table 1. In three experiments we observed a transformation rate ranging from 0.07 to 0.1 following a dose of 300 rad. This frequency is about tenfold lower than that obtained in earlier *in vitro* experiments. It is closer to the frequency observed in some of the studies carried out *in vivo*[6].

Several intriguing questions are evoked. Does the host mediate in modulating transformation by radiation? Is there a repair of transforming events before they can be expressed? How significant is the state of cells during irradiation in determining the rate of transformation? It is well known from *in vitro* studies that cell replication is required for fixation of the transformation[20,4,5]. With the *in vitro* technique, cells are seeded as single cells with ample opportunity to divide. In addition, of course, they are not in contact with one another, and constitute a mixture of cell types from many tissues. *In utero* the situation is quite different; the embryonic cells are irradiated as tissues where there is cell to cell contact in

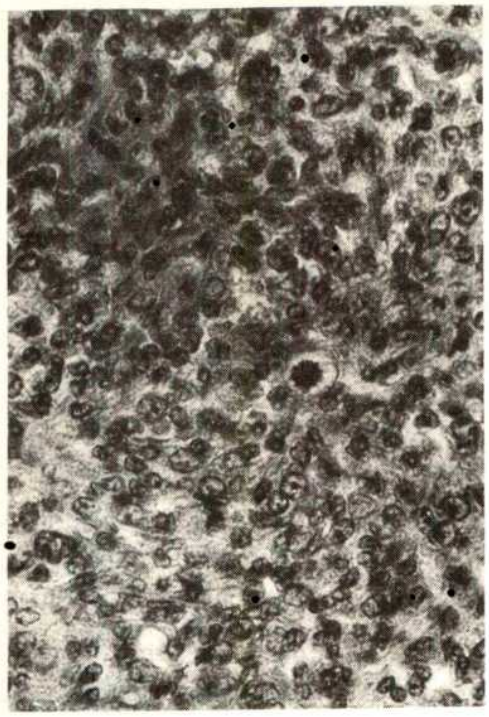

Fig. 2 Section of 3-week-old malignant invasive carcinoma growing in a Syrian hamster following s.c. injection of 5 × 10⁵ cells of a cell line (LSH 5) transformed *in utero* by X-ray irradiation (× 220).

tissue-specific arrangements, and where the rate of cell replication varies with the tissue. It remains to be seen which of these factors, if any, is responsible for the lowered yield of transformed cells characteristic of *in utero* as opposed to *in vitro* irradiation.

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Local inhibition of centripetal particle transport where LETS protein patterns appear on 3T3 cells

WE try here to establish a link between a motility phenomenon in animal cells and the presence of the so-called 'large external transformation-sensitive (LETS)' protein on the surface of the cells. Through studies of this protein a new and still puzzling aspect of animal cell surfaces became apparent. Originally discovered as a major surface glycoprotein of molecular weight 220,000–250,000 which disappeared on cell transformation (for review, see ref. 1), it has since become quite likely that various differently named surface glycoproteins—galactoprotein a (ref. 2), fibroblast surface antigen[3], Zeta protein[4], 'cell-surface protein' (CSP)[5], and 'cold-insoluble globulin' (CIG)[6–8]—which were studied independently by different investigators, are identical to or at least very closely related to LETS protein.

If studied by the methods of indirect immunofluorescence, the distributions of LETS protein on the dorsal surface of fibroblastic cells show intriguing aspects. The protein distribution patterns, or at least their antigenic availability, seem to originate from cell–cell contact areas[9]. Later on, the protein can cover large cell surface areas in the form of streaks, indicating a major inhomogeneity in the cell surface composition. Furthermore, it does not remain localised on the cell which produced it. In several-day-old cultures it can form three-dimensional fibrous networks which span between cells throughout the whole culture. The biological function of this protein is not yet entirely clear; however, it has been found to restore normal cell morphology in transformed cells[10], to correlate inversely with tumorigenicity[9], and to change from a dense fibrillar network in prefused myoblasts (Yaffe's rat myogenic cell line L8) to a sparse dot pattern in myotubes[11].

The centripetal surface flow[12–15], that is, the radial transport of particles attached to the cell surface along that surface at speeds of 0.2–3 μm min^{-1} towards the perinuclear region in cultured fibroblastic or epithelial cells (see Fig. 2), is another quite puzzling surface phenomenon. It is not restricted to the cell body but is also expressed on the surface of lamellipodia, blebs and even in the only 0.2-μm thin needle-shaped filopodia[16,17]. At present, neither a molecular mechanism nor a cellular structure are known which could explain this unidirectional and radial particle movement. Although it is generally believed that the whole cell surface flows constantly towards the nucleus[15,21], it is not yet ruled out that the particle movement may be induced only locally at the site of encounter between the cell surface and non-cellular material.

Assuming a constant centripetal flow, one might expect that fibrillar surface components like LETS protein eventually have to orient themselves parallel to the radial flow lines. The complex distribution patterns of LETS protein of all cell lines investigated so far seemed hard to reconcile, however, with a radial centripetal flow (see Fig. 1). One may suspect, therefore, that the flow has ceased in areas covered with LETS protein.

Indeed, the observation of movements of fluorescent antibody-labelled LETS protein patterns in live 3T3 cells showed only slow distortions of the fibre arrangement (Fig. 1). LETS protein fibres could stretch, shorten, fold, move towards or away from the nucleus; however, the combined movements seemed to be unidirectional and very slow. We also observed gold particles of 2,000–4,000 Å diameter (particle clusters up to 1 μm diameter)[16,17] which had attached to the dorsal cell surfaces inside an area covered with LETS protein, and did not find that they moved centripetally.

One may argue that these experiments do not reflect the natural cell surface movement any more, because the antibody treatment, necessary for the visualisation of LETS protein patterns, influenced the cells. Therefore, we allowed 3T3 cells to first centripetally transport the gold particles, and subsequently detected the LETS protein on the dorsal cell surface by indirect immunofluorescence with CIG antibody. Figure 2 shows in the case of isolated cells two stages of the radial and centripetal movement of the particles from the cell edge where they were picked up, towards the perinuclear region (Fig. 2*a*) where they accumulated and eventually became internalised (Fig. 2*b*). It took 4–6 h before 10–20% of the cells had accumulated sufficient particles to show recognisable particle rings around the nucleus. The rings of particles (Fig. 2*a*) were not always completely closed, not even in isolated cells.

Four hours after exposure to gold particles—that is, before the particles became internalised, we fixed 3T3 cell cultures on glass coverslips and stained them for LETS protein. The result is shown in Fig. 3. Cells which were able to accumulate the gold particles all round the nucleus in the form of a ring showed only a few patches or no LETS protein at all (Fig. 3*a*, cell a), whereas cells with extended patterns of LETS protein still had the particles scattered randomly all over their surface (Fig. 3*a*, cells b and c). We also observed quite a few cells with incomplete gold rings. In many such cases the ring segment marked a cell-surface sector without LETS protein, whereas the complementary sector contained extended LETS protein patterns (Fig. 3*b*). We never found a big particle cluster on top of an extended LETS protein pattern. Clusters were often located, however, at the edge of a pattern.

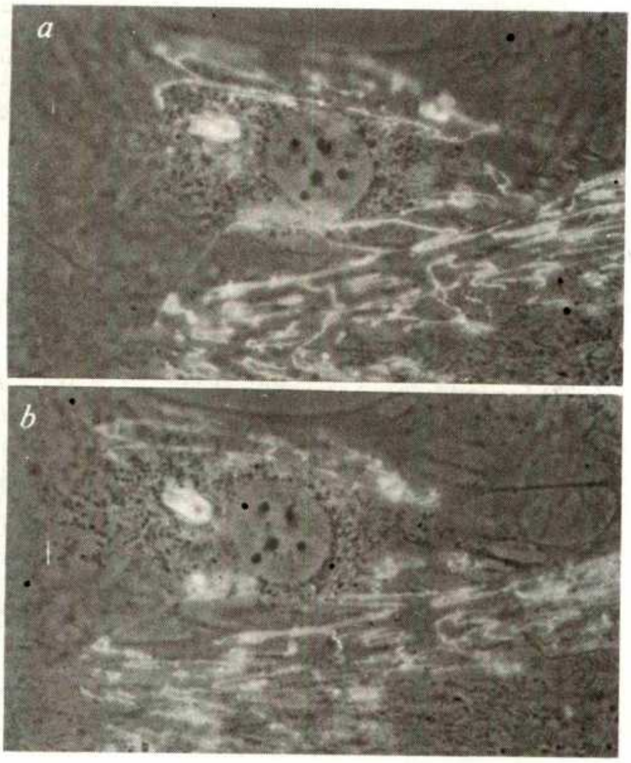

Fig. 1 Movements of LETS protein patterns on live 3T3 cells. Bar indicates 10 μm. 3T3 cells grown for 3 d on glass coverslips in DME + 10% calf serum were washed in DME, incubated at 37 °C for 15 min in rabbit anti-cold-insoluble globulin antibody (anti-CIG antibody) in DME (dilution 1:80). Anti-CIG antibodies bind to LETS protein[18] and also stain the same cellular structure as anti-LETS antibodies[6,9,11]. CIG antigen purification and antibody preparation were as described previously[9,11]. After a further washing in DME, the coverslips were incubated for 15 min at 37 °C in DME containing fluoresceinisothiocyanate-labelled goat anti-rabbit IgG (Meloy, Springfield, Virginia; dilution 1:20), washed again, and returned to DME + 10% calf serum. Live cells were observed in a Zeiss Photomicroscope III (63x; N.A. 1.4) as described previously[22]. *a*, LETS protein pattern on the dorsal surfaces of two adjacent 3T3 cells. Pictures were taken such that a phase-contrast image was created by the illumination from below the stage while an epi-illuminator produced and superimposed the fluorescent image. *b*, Same pattern 48 min later.

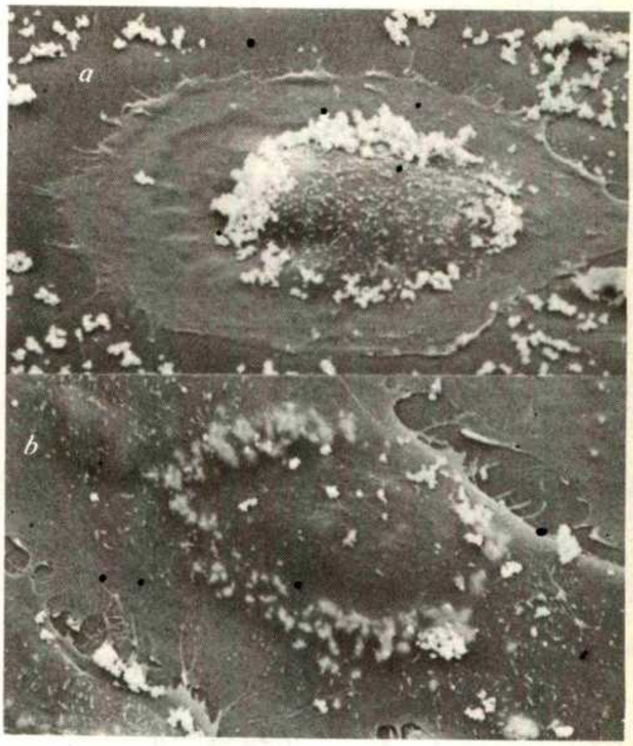

Fig. 2 Centripetal transport of gold particles in 3T3 cells. (Scanning electron microscopy; bar indicates 10 μm in horizontal direction.) Swiss 3T3 cells (Dr H. Green, MIT) were grown for 2–3 d on glass coverslips in DME supplemented with 10% calf serum. After adjusting a colloidal suspension of gold particles[16,17] to 290 mOsm by adding 49 mg ml^{-1} D-glucose (Baker, Phillipsburg, New Jersey), the 3T3 cell cultures were rinsed in PBS and incubated for 5 min in gold suspension at 37 °C. During this time the particles settled on top of the substrate and dorsal cell surfaces. The density of adherent particles is much higher in the substrate than on the cell surfaces. After exposure to gold particles, the coverslips were washed, incubated again in DME + 10% calf serum and returned to the incubator. Cells of cultures which are 3 d old or younger often retracted slightly after the staining procedure, but reverted rapidly to normal morphology. *a*, Isolated 3T3 cell with a complete ring of particles (arrow) 4 h after exposure to the staining procedure (tilt angle 60°). *b*, Another cell 22 h after staining. Between 12 and 22 h the rings of particles become internalised, as seen by the blurred image of the ring which is now below the cell surface (tilt angle 45°).

These observations suggest that the particles cannot move radially into or inside areas where LETS protein covers the cell surface in the form of extended patterns. One would expect, therefore, that removal of LETS protein from the cell surface would increase the centripetal transport of particles.

During a 10-min treatment of a 3T3 cell culture with 5 μg ml^{-1} trypsin in Dulbecco's Modification of Eagle's medium (DME), the predominant component removed from the dorsal cell surfaces is LETS protein[4,19,20]; yet the cells show no detectable shape alteration in response to this treatment. Therefore, 3T3 cells, grown for 5 d on glass coverslips, were washed in DME and incubated at 37 °C for 10 min in DME containing 5 μg ml^{-1} trypsin (Miles Laboratories, Kankahee, Illinois) and then returned to their previous culture medium for 45 min. Control cultures were similarly treated with DME or DME containing 5 μg ml^{-1} thrombin (Dr J. Fenton, Albany, New York) since it has been shown earlier that neither treatment affects the LETS protein[20]. Subsequently, the cells were exposed to gold particles as described in Fig. 2, and returned to their previous culture media for 4 h. After fixation of the preparations, we determined the percentage of cells which had accumulated particle clusters. In 20 randomly selected fields we counted

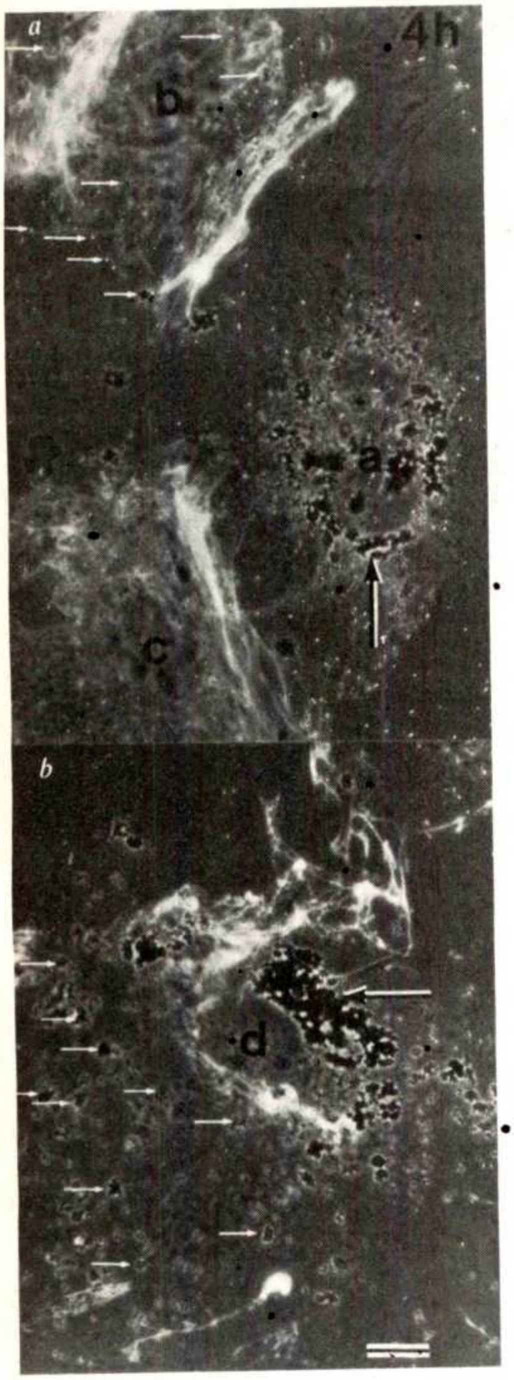

Fig. 3 Mutual exclusion of extended LETS protein patterns and areas with centripetal particle transport (bar indicates 10 µm). 3T3 cells were grown for 5 d without medium change on 12 × 12-mm glass coverslips and incubated in gold particle suspension as described in Fig. 2. Four hours later, the cells were fixed in 3.5% formaldehyde in PBS and incubated for 30 min at 37 °C with anti-CIG antibody in PBS (dilution 1:80). After a further washing in PBS, the coverslips were incubated for 30 min in DME containing fluoresceinisothiocyanate-labelled goat anti-rabbit IgG (dilution 1:20), washed again, mounted and observed in the same conditions as described in Fig. 1. *a*, 3T3 cell (a) with a complete ring of gold particles (arrow) and no LETS protein on the surface, and two other cells (b, c) with extended LETS protein nets but no particle ring. Randomly scattered small particle clusters are indicated by small white arrows. Big particle clusters appear black. *b*, Incomplete particle ring (arrow) with LETS protein patterns covering the complementary ring segment. Opposite to the ring a 'barrier' of LETS protein can be seen and behind it many particle clusters (arrow points to one of them), which were not moved towards the nucleus (d).

approximately 300 cells in each preparation and found the following percentages of cells with particle clusters. (Standard deviations describe variations over the selected fields). DME + 5 µg ml^{-1} trypsin: 64% (s.d. 15%); DME + 5 µg ml^{-1} thrombin: 20% (s.d. 8%); DME: 13% (s.d. 10%). The three- to four-fold increase in particle accumulation by the trypsin-treated cells compared with controls suggests that removal of LETS protein from the cell surfaces does increase the speed and extent of the centripetal particle transport around the cells' perimeters.

It seems, therefore, that the centripetal transport of surface-attached particles and, perhaps, the surface flow itself are locally inhibited by those surface networks which are predominantly built of LETS protein. It remains to be seen whether there is an inhibitory effect of LETS protein on cell motility in general, as was originally postulated by Hynes[23]. In view of the observation that LETS protein networks seem to originate from cell–cell contacts, the possibility that those networks inhibit surface flow seems to offer an intriguing mechanism of cell–cell interaction: It would suggest a way by which the surface movements in two or more cells become altered depending on direction and extent of their mutual contacts.

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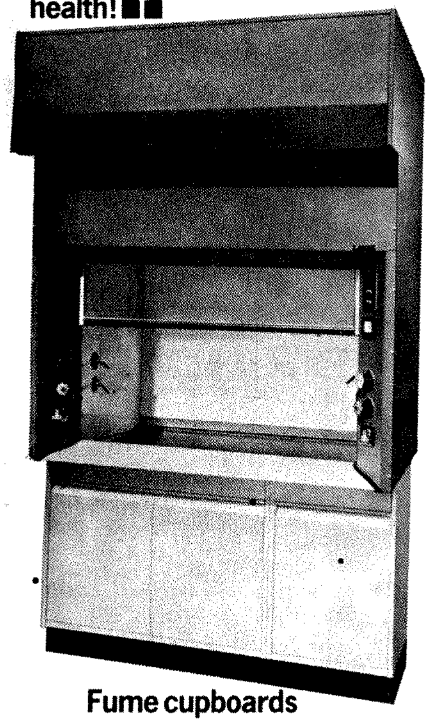

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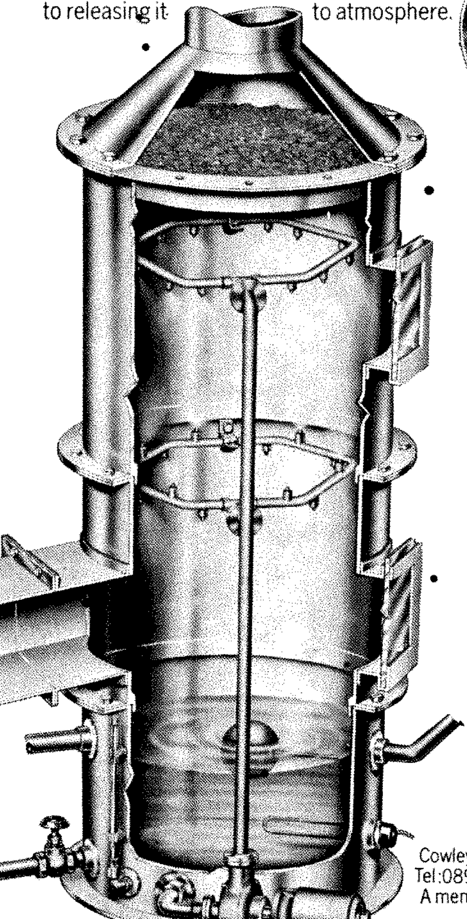

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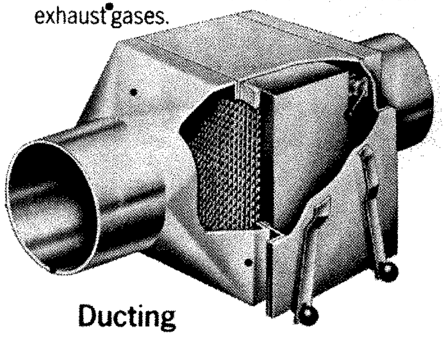

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JOE D. BURCHFIELD

A controversial figure during his lifetime, Lord Kelvin's wide-ranging interests led him to become involved in a public dispute on the validity of the doctrine of uniformitarianism in geology.

Equally at home with both the abstract theories and the practical applications of science, Kelvin was an accomplished mathematician, a successful engineer, and a scientific theorist whose interests ranged over every branch of physics including physical astronomy and physical geology.

This book discusses Kelvin's influence on late Victorian geology, his passionate belief that uniformitarianism ignored the established laws of physics and that a great reform was needed to return geology to the path of true science. It describes the disputes in which Kelvin's beliefs involved him with such eminent scientists as Darwin, Huxley and Wallace, and how his theories influenced their work.

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particular target[1–3,6,7]. Similarly, union of effectors with targets by means of a different receptor such as the complement receptor may not result in cytotoxicity[8,9]. Whether the efficiency of different cells at mediating ADCC depends on the number, type or affinity of Fc receptors, whether a cooperation between different types of surface receptors is needed[10] or whether some property of the target cells decides the outcome of contact between effectors and targets, has not been established. It is likely that ADCC occurs only if a sufficient union is established between effector and target cell. Such a union could be generated by bonds between the target cell and two types of surface receptors on the effectors—such as the Fc receptor and the complement receptor, both of which are present on effector cells[10–12]. Using herpes-virus infected target cells we here provide support for this hypothesis and show that the addition of complement, at levels below those required for antibody–complement lysis, considerably enhances

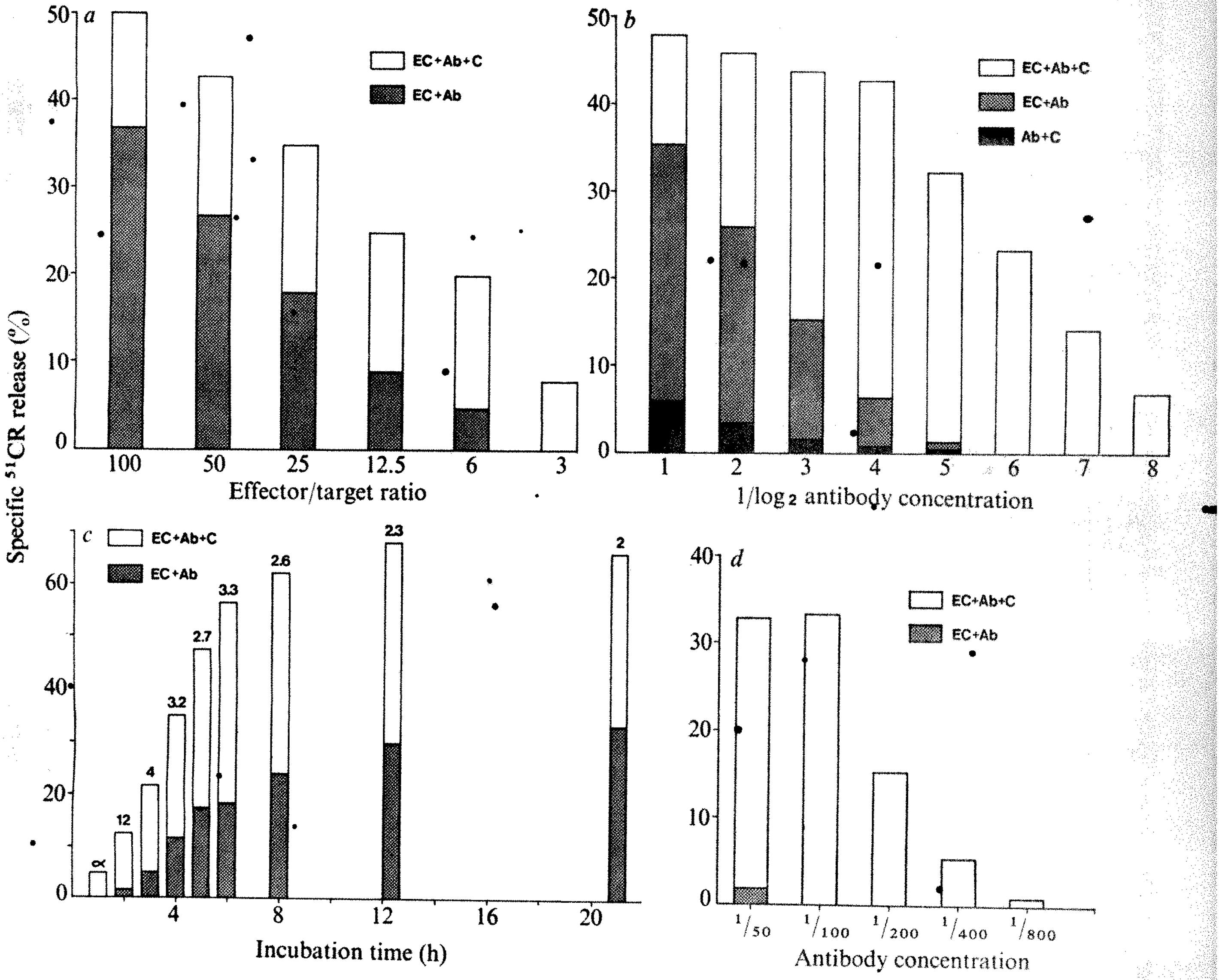

Fig. 1*a*, Enhancement of neutrophil-mediated ADCC at various effector-to-target cell ratios by rabbit complement. Neutrophils were collected from the bovine mammary gland and purified[14]. Target cells were infected with IBR virus and labelled with $Na_2\ ^{51}CrO_4$ 16 h before the start of assays[6]. Target cells were sensitised with a 1/800 final concentration of hyperimmune bovine anti-IBR serum. Rabbit complement was added at a final concentration of 1/70. At this concentration, lysis of antibody-sensitised target cells in the absence of effector cells was 2% above background (12% in a 6-h assay). Assays were performed in microtitre trays[5]. The percentage specific ^{51}Cr release was computed by the formula

$$\text{specific release (\%)} = \frac{\text{radioactivity in test supernate} - \text{control}}{\text{total releasable radioactivity} - \text{control}} \times 100.$$

The neutrophils in the absence of anti-IBR serum failed to give release of ^{51}Cr above background either in the presence of media alone or media plus 1/70 rabbit complement. EC, effector cells; Ab, anti-IBR serum; C, complement, *b*, Enhancement by complement of neutrophil-mediated ADCC at different concentrations of anti-IBR serum. Assay as for (*a*). The assay was run at a neutrophil/target cell ratio of 100:1. The final antiserum concentration at level 1 was 1/100 and 8, 1/12,800. Rabbit complement was included at a final concentration of 1/70. *c*, Enhancement by complement of neutrophil-mediated ADCC at different durations of assay. The neutrophil/target cell ratio was 100:1, the final concentration of sensitising anti-IBR serum 1/800 and rabbit complement 1/70. Figures at the top of each column refer to the magnitude of the specific release in the presence of complement compared to that without complement. The background releases ranged from 6.7% at 1 h to 18.4% at 21 h in media alone and from 8.9% at 1 h to 22% at 21 h in the presence of complement. *d*, Enhancement of lymphocyte-mediated ADCC by rabbit complement. Lymphocytes were collected from peripheral blood by Ficoll–Hypaque flotation and were further purified by passage over glass wool to remove adherent cells[13]. The preparation gave a low level of specific ^{51}Cr release (2%) at the highest concentration of serum tested, possibly due to some remaining macrophages or neutrophils. The assay was performed for 6 h in the presence or absence of 1/70 final concentration of rabbit complement. The level of specific release by complement in the absence of effector cells was 5% at 1/50, 3% at 1/100 and 2% at 1/200 of anti-IBR serum, respectively.

ADCC and, furthermore, permits cells to mediate ADCC that in the absence of complement could not do so.

Previously, we have described the cell types and conditions necessary to demonstrate ADCC against bovine antibody sensitised bovine target cells infected with infectious bovine rhinotracheitis (IBR) virus—a bovine herpesvirus[6]. We have compared several cell types and have shown that neutrophils perform best and that lymphocytes fail to mediate ADCC[13]. Lymphocytes, however, do show activity against erythrocyte target cells[6]. We hypothesised that since the cells that mediate ADCC had complement receptors as well as Fc receptors[11,12], then if bonds between effectors and targets were additionally engaged by the complement receptor then cytotoxicity should be enhanced. Such enhancement should be more apparent in those conditions where cytotoxicity was submaximal, such as low effector-to-target cell ratios, low concentrations of sensitising antiserum and short term assays. That the addition of complement does enhance ADCC is shown in Figs 1–3. Also shown are data that support the predictions—enhancement was more apparent at low effector-to-target cell ratios (Fig. 1*a*), low concentrations of sensitising antiserum (Fig. 1*b*) and short duration assays (Fig. 1*c*). One explanation for the enhanced cytotoxicity could be that in addition to ADCC we were also observing antibody-complement mediated cytotoxicity. This possibility was considered remote since the levels of complement used in the experiments either failed to exhibit or gave only low levels of cytotoxicity against targets in the absence of effector cells. At high complement concentrations, however, such antibody-complement cytotoxicity was observed, indicating that the material did possess complement activity (data not shown). Furthermore, the rabbit complement preparation was not providing an additional sensitising antibody since heat inactivated material failed to give enhancement.

Not only could the addition of complement lead to enhancement of cytotoxicity, but cell types incapable of mediating ADCC against the virus-infected cells could be made to do so by the addition of complement. Thus lymphocytes alone could not mediate ADCC (Fig. 1*d*), but ADCC became readily apparent on addition of complement.

These experiments support the hypothesis that multiple binding of effectors to target cells will be followed by enhanced cytotoxicity. It needs to be established if the effect is the result of a more tenacious bond or has some other explanation such as the generation of a biochemical signal that results in target cell lysis. Finally, since ADCC has been considered as an *in vitro* model for an antiviral mechanism of defense[6], the implications of our results should be considered in terms of the possible part that complement might have in enhancing recovery from certain viral infections.

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Selective incorporation of H-2 antigenic determinants into Friend virus particles

IN susceptible mice, Friend murine leukaemia virus (FV) produces fatal erythroleukaemia[1] characterised by rapid onset of splenomegaly and viraemia in 4–5 d and leading to the death of the animal in as little as 3–4 weeks. In the replication of this enveloped, C-type RNA virus, assembly occurs at the surface of infected cells where the particles mature by budding[2]. We have examined the question of whether host cell surface glycoproteins are incorporated into the virion as the host cell membrane becomes part of the completed particle during the budding process. In particular, serologically identifiable alloantigens governed by the $H\text{–}2K$ and $H\text{–}2D$ genes of the major histocompatibility complex and found on cell surfaces have been sought in lysates of serum-derived FV.

Serum collected from mice infected 10–14 d previously with a high dose of NB-tropic FV was the source of virus. After clarification of the serum at 6,800*g*, virus was pelleted by centrifugation at 100,000*g*. The pellet was resuspended in buffered saline containing detergent (Nonidet P-40, 0.25%) to disrupt the virus. This preparation was then examined for the presence of $H\text{–}2K$ and $H\text{–}2D$ antigens of the host mouse by determining its capacity to inhibit the cytotoxic activity of various anti-$H\text{–}2$ antisera. Figure 1 shows the results of an experiment with virus collected from the serum of FV-infected (BALB/c × BALB.B)F_1 mice ($H\text{–}2^{\mathrm{b}}/H\text{–}2^{\mathrm{d}}$). The preparation was tested for its capacity to inhibit the complement-mediated lysis of uninfected (BALB/c × BALB.B)F_1 lymph node target cells with antisera monospecific for either $H\text{–}2K^{\mathrm{b}}$, $H\text{–}2D^{\mathrm{b}}$, $H\text{–}2K^{\mathrm{d}}$ or $H\text{–}2D^{\mathrm{d}}$ antigenic specificities (Table 1). Only lysis by anti-$H\text{–}2D^{\mathrm{b}}$ antiserum {(A × HTI)F_1 anti-EL4; $H\text{–}2^{\mathrm{a}}/H\text{–}2^{\mathrm{i}}$ anti-$H\text{–}2^{\mathrm{b}}$} was detectably inhibited (Fig. 1*a*). An identical pattern of inhibition was seen using a second anti-$H\text{–}2D^{\mathrm{b}}$ antiserum (BALB/c anti-BALB.G; $H\text{–}2^{\mathrm{d}}$ anti-$H\text{–}2^{\mathrm{g}}$) raised against normal spleen cells rather than EL4 tumour cells. Similarly, in virus grown in (C57BL/6 × DBA/2)F_1 mice, which are also $H\text{–}2^{\mathrm{b}}/H\text{–}2^{\mathrm{d}}$ heterozygotes but with a different genetic background, $H\text{–}2D^{\mathrm{b}}$ alloantigen but not $H\text{–}2K^{\mathrm{b}}$, $H\text{–}2K^{\mathrm{d}}$ or $H\text{–}2D^{\mathrm{d}}$ was detected. Neither sham virus preparations made from non-viraemic BALB.B mouse serum nor preparations made from viraemic BALB/c mouse serum produced inhibition of lysis by any of the four antisera (data not shown).

Table 1 Anti-H-2 antisera characteristics

	Alleles recognised	Private specificity
(A × BALB.B) F_1 anti-BALB/c	$H\text{–}2K^{\mathrm{d}}$, $H\text{–}2I^{\mathrm{d}}$	H-2K.31
BALB.G anti-BALB/c	$H\text{–}2D^{\mathrm{d}}$	H-2D.4
(A × BALB.G) F_1 anti-BALB.B	$H\text{–}2K^{\mathrm{b}}$, $H\text{–}2I^{\mathrm{b}}$, $H\text{–}2S^{\mathrm{b}}$	H-2K.33
(A × HTI) F_1 anti-EL4	$H\text{–}2D^{\mathrm{b}}$	H-2D.2
BALB/c anti-BALB.G	$H\text{–}2D^{\mathrm{b}}$	H-2D.2

Several criticisms of the experimental techniques used are in order. The augmentation of lysis in the presence of virus above controls is difficult to rationalise (Fig. 1*b*, *c* and *d*). It is not due to the NP-40 detergent, since controls contain identical amounts of detergent. A contaminant in the 100,000*g* pellet may be responsible, because no such augmentation of lysis was observed in recent preliminary experiments using sucrose density-gradient purified virus. Contamination was not the cause of inhibition of anti-$H\text{–}2D^{\mathrm{b}}$-mediated cytolysis, however, for three reasons. First, inhibition of lysis by contaminating plasma membranes would be expected to produce inhibition of all four antisera. Second, BALB.B ($H\text{–}2^{\mathrm{b}}$) non-viraemic serum preparations were not inhibitory. And third, BALB/c ($H\text{–}2^{\mathrm{d}}$) virus preparations were also not inhibitory. Thus, $H\text{–}2D^{\mathrm{b}}$ was the

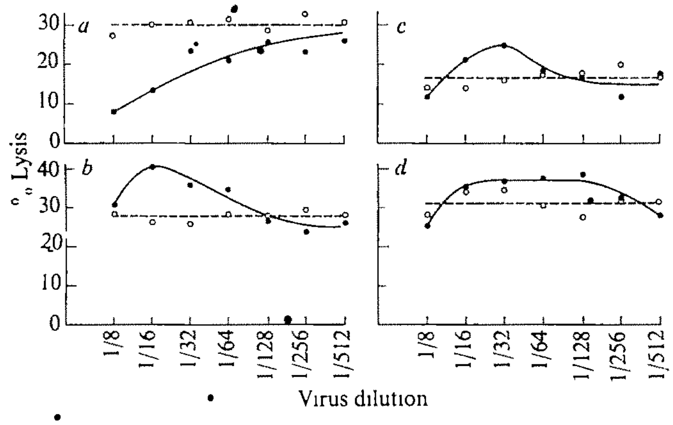

Fig. 1 Serum, prepared from blood collected from (BALB.B ×BALB/c)F_1 mice given 10^4 spleen focus forming units of Friend virus 10–14 d previously, was centrifuged at 6,800*g* for 15 min and the supernatant recovered After re-centrifugation of the serum at 100,000*g* for 90 min, the supernatant was discarded and the pellet resuspended in 0 1 ml phosphate-buffered saline (PBS) containing 1mM EDTA to which was added 0.01 ml of a 2.75% (v/v) solution of NP-40. After 1 h incubation at 4 °C, 0 11 ml of a 25% (w/v) bovine serum albumin (BSA) solution was added to neutralise the NP-40 and the entire mixture diluted 1:8 with medium 199 containing 20% foetal calf serum. Control preparations, lacking viraemic serum pellets, contained only PBS, EDTA, NP-40, BSA and 199 with 20% FCS in proportions identical to virus preparations. One-tenth ml of either virus or control preparations was aliquoted into 5-ml Falcon plastic culture tubes in serial twofold dilutions. To these was added 0.1 ml of monospecific anti-*H–2* antiserum appropriately diluted in 199 with 20% FCS After 10 min incubation at room temperature, 0 05 ml of ^{51}Cr-labelled (BALB.B×BALB/c)F_1 mesenteric lymph node cells (10^6 ml^{-1}) was added followed by the addition of 0 05 ml of neat guinea pig serum as a complement source. After 1 h at 37 °C in a 5% CO_2 atmosphere, 0.3 ml of PBS containing 10 mM EDTA was added to terminate the reaction, the cultures spun at 300*g* for 5 min and 0.3 ml of supernatant recovered for counting γ radiation Curves were generated in the presence of either virus preparations (●) or control preparations (○). Anti-*H–2* antisera were raised by the alloimmunisations—*a*, (A×HTI)F_1 anti-EL4, $H{-}2^{a}/H{-}2^{i}$ anti-$H{-}2^{b}$—anti-$H{-}D^{b}$ antiserum; *b*, (A×BALB.G)F_1 anti-BALB.B, $H{-}2^{a}/H{-}2^{g}$ anti-$H{-}2^{b}$—anti-$H{-}2K^{b}$, *c*, BALB.G anti-BALB/c, $H{-}2^{g}$ anti-$H{-}2^{d}$—anti-$H{-}2D^{d}$, and *d*, (A×BALB.B)F_1 anti-BALB/c, $H{-}2^{a}/H{-}2^{b}$ anti-$H{-}2^{d}$—anti-$H{-}2K^{d}$.

sole antigen detected, and it was only detected in virus-containing preparations from mice with the $H{-}2D^{b}$ allele.

H–2 antigen activity of host cell specificity has been detected before in an enveloped RNA virus. Hecht and Summers[3] observed inhibition of cytolysis mediated by anti-$H{-}2^{k}$ antisera in the presence of highly purified vesicular stomatitis virus grown in L cells ($H{-}2^{k}$). Because of the purification techniques used, they concluded that the *H–2* antigens were an integral part of the virion. Their finding that both $H{-}2K^{k}$ and $H{-}2D^{k}$ antigens were present in the virion could be explained by the random distribution of *H–2* molecules on plasma membrane destined to become virus envelope. Our finding of only one of four host cell *H–2* specificities in FV suggests that the inclusion of *H–2* antigens in virus particles is selective rather than random.

The $H{-}2^{b}$ haplotype confers relative resistance to FV disease[4]. One gene responsible for this resistance, *Rfv*-1, maps in or near the $H{-}2D^{b}$ region[5] Resistance in BALB.B mice results in strong rejection of implanted cells of syngeneic FV-induced tumour lines, whereas BALB/c mice resist implants of syngeneic FV tumour cells poorly[6]. Characteristic of resistance in BALB.B but not BALB/c mice is the appearance of cytotoxic T lymphocytes specific for the syngeneic tumour. The lytic activity of these BALB B T cells *in vitro* is subject to *H–2* restriction; that is, lysis can only be demonstrated against target cells expressing both FV and $H{-}2^{b}$ antigens[7].

Present evidence for the basis of *H–2* restriction in this and other T-cell killing systems supports the hypothesis that *H–2* molecules associate with virus molecules on the target cell surface in such a way as to serve as an 'altered-self' marker recognisable by the immune T lymphocyte[8]. Evidence in favour of a physical association at the cell surface between virus and *H–2* molecules has been obtained in this laboratory with the demonstration that capping of FV antigens on $H{-}2^{b}$ tumour cell surfaces causes partial co-capping of *H–2D* but not *H–2K* antigens[9].

If, as the evidence indicates, *H–2* antigens physically associate with virus antigens on the cell surface, then the appearance of *H–2* in the virion is most likely the consequence of such an association at the site of virus budding. The inclusion of only one of four possible *H–2* antigens in the virus, however, implies that not all *H–2* molecules are equal in their capacity to bind to cell surface virus antigens. The selective inclusion of *H–2* molecules in C-type virus particles is in keeping with the sporadic detection during biochemical analysis of MuLV of a glycoprotein[10] of 45,000 molecular weight. The molecular weight and irregular detection of this constituent suggests that it may consist in part of host cell *H–2*, inclusion of which in virions depends on its capacity to bind to virus molecules.

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Prevention of experimental allergic encephalitis in Lewis rats by rat and bovine spinal cord proteins

EXPERIMENTAL allergic encephalitis (EAE) is an inflammatory autoimmune disease of the central nervous system (CNS) that is mediated by T lymphocytes sensitised to the organ-specific basic protein (BP) of CNS myelin[1]. Lesions of delayed hypersensitivity develop in the brain and spinal cord when sensitised lymphocytes traverse the walls of CNS blood vessels and react with the target antigen in myelin[1]. EAE can be prevented by injecting BP in Freund's incomplete adjuvant (FIA) before challenge, suppressed by injecting BP soon after challenge and treated by injections of BP after clinical symptoms appear[4]. Prevention, suppression and treatment are immunologically specific for BP and can be dissociated from the production of humoral antibody against BP[5]. The demonstration that pretreatment of guinea pigs with bovine spinal cord protein (BSCP)[6,7] prevented the development of EAE[8,9] is thus of considerable importance because BSCP is chemically and antigenically distinct from bovine BP and is not encephalitogenic. As pretreatment with SCP induced a state of nonspecific unresponsiveness to bovine BP in the guinea pig, we investigated whether SCP had anti-encephalitogenic activity in the Lewis rat, a highly inbred rat strain used extensively for the study of EAE. We report here that Lewis rats pre-

Table 1 Encephalitogenicity of rat BP, RSCP and BSCP in Lewis rats

Antigen used for challenge* (in FCA)	Amount of antigen injected (μg)	EAE			
		No. sick / No. tested	Onset (day)	Clinical severity†	Histologic severity ‡
Rat BP	20	6/6	14.5	1.8	1.5
Rat BP	30	7/7	12.5	3.4	1.7
Rat BP	50	10/10	13	3.0	1.8
RSCP	300	0/6	—	0	0
BSCP	400	0,8	—	0	0

*Animals were injected in the left hind foot pad with 0.1 ml of an inoculum prepared by emulsifying the appropriate amount of antigen in 0.05 ml of saline with 0.05 ml of FIA containing 200 μg of *M. butyricum*.

†Average scores for clinical symptoms were graded as follows; +, transient weight loss; ++, limp tail; +++, hind end weakness and/or incontinence; ++++, complete loss of tone in tail (string tail) and paralysis.

‡Average score for lesions in brain and spinal cord graded from 0 to 2+ according to Levine *et al.*[12].

treated with rat SCP (ref. 10) were protected against EAE, as they did not develop the disease when they were subsequently immunised with rat myelin BP in complete Freund's adjuvant (CFA).

Homologous RSCP was investigated first because immunodiffusion studies with anti-BSCP serum and anti-RSCP serum indicated that bovine, monkey, human and rabbit SCP were antigenically closely related[7,10], while RSCP was significantly different. The homologous rat BP was used for the induction of EAE. RSCP was purified from rat spinal cord[10]. Rat BP containing both the L and the S BP was prepared from rat brain by the method of Deibler *et al.*[11].

Male Lewis rats weighing about 250 g were injected in the right hind foot pad at different intervals with 0.1 ml of inoculum prepared by emulsifying the required amount of RSCP with an equal volume of FIA. Control rats were not treated or were injected with bovine gamma globulin (BGG) emulsified with FIA. At the appropriate time, the animals were challenged by injecting 0.1 ml of an FIA emulsion containing 30 μg of rat BP and 200 μg of *Mycobacterium butyricum* into pads in the left hind foot.

Table 1 shows that 20 μg of rat BP was sufficient to produce clinical and histological EAE in all animals tested. Disease onset usually occurred 15 d after challenge. Higher doses of rat BP induced more severe disease with onset 12–13 d after challenge. The 30 μg of rat BP used to induce EAE in all pretreated animals was selected as a moderately severe challenge dose. Neither RSCP nor BSCP were encephalitogenic in Lewis rats at levels of 300 and 400 μg, respectively.

Results of pretreatment schedules in which the total amount of RSCP used varied from 300 to 900 μg, and the pretreatment interval extended from 5 to 21 d are recorded in Table 2. Pretreatment with three weekly doses of 100 μg of RSCP protected five out of six animals from clinical and histological EAE. Doubling or tripling the amount of RSCP injected over 2 or 3 week intervals gave complete protection of all animals, while control animals that received similar amounts of BGG were not protected.

Table 2 Prevention of EAE* in Lewis rats by pretreatment with RSCP—effect of variations in dosage and pretreatment schedule

Pretreatment (in FIA)			EAE			
Schedule	Days†	Total dose (μg)	No. sick / No. tested	Onset (day)	Clinical severity‡	Histologic severity§
1 × 100 μg RSCP weekly	21	300	1/6	14	0.3	0.5
2 × 100 μg RSCP weekly	21	600	0/6	—	0	0.2
2 × 100 μg BGG weekly	21	600	6/6	12.5	3.1	2.0
3 × 100 μg RSCP weekly	21	900	1/6	15	0.3	0.2
3 × 100 μg BGG weekly	21	900	5/6	12	2.1	0.7
2 × 200 μg RSCP weekly	14	800	0/6	—	0	0
2 × 200 μg BGG weekly	14	800	4/4	13.5	2.7	2.0
3 × 100 μg RSCP	10	300	5/6	13.0	2.8	2.0
3 × 100 μg BGG	10	300	6/6	11.5	3.0	2.0
3 × 100 μg RSCP	5	300	6/6	12.0	3.0	2.0
None			10/10	11.5	2.9	1.9

*Animals were challenged with 0.1 ml of inoculum containing 30 μg of rat BP and 200 μg of *M. butyricum*.

†Number of days between first pretreatment injection and challenge.

‡Average score for clinical symptoms graded 0 to 4+.

§Average score for lesions in brain and spinal cord graded from 0 to 2+.

Table 3 Prevention of EAE* in Lewis rats by pretreatment with different amounts of bovine SCP for 21 d

(Pretreatment (in FIA)		EAE			
Schedule	Total dose (μg)	No. sick/ no. tested	Onset (day)	Clinical severity†	Histologic severity‡
2 × 83 μg BSCP weekly	500	5/6	12	3.0	1,8
2 × 170 μg BSCP weekly	1,000	5/6	14	2.0	1.0
2 × 340 μg BSCP weekly	2,000	1/6	—	0.5	0.2
2 × 170 μg BGG weekly	1,000	5/5	11	3.6	2.0
None		6/6	10.5	3.5	2.0

*Animals were challenged 3 d after the last pretreatment injections with 0.1 ml of inoculum containing 30 μg rat BP and 200 μg *M. butyricum* emulsified in FIA.

†Average score for clinical symptoms graded 0 to 4+.

‡Average score for lesions in brain and spinal cord graded from 0 to 2+.

When the pretreatment intervals were shortened to 10 or 5 d, however, three 100-μg doses of RSCP did not protect. The results suggest that the amount of RSCP needed for protection is inversely related to the length of the pretreatment period.

Heterologous BSCP also had the capacity to protect Lewis rats against EAE induced with rat BP, although approximately seven times as much BSCP was required to achieve the same degree of protection provided by 300 μg of RSCP (Table 3). The fact that RSCP and BSCP prevent EAE in the rat as well as in the guinea pig suggests that SCP may be used as a nonspecific anti-encephalitogenic agent

One possibility that can probably be ruled out is that of specific immunosuppression due to contamination of RSCP with rat BP because SCP was extracted from bovine or rat spinal cord (not de-fatted) with 0.05 M acetate buffers, a procedure that does not solubilise detectable amounts of BP. Furthermore, the amount of rat BP in 1.0 mg of purified RSCP was found not to exceed 30 ng by a competitive inhibition radioassay (unpublished results). This method measures the extent to which binding between a known amount of ^{125}I-rat BP and a fixed volume of a standard rabbit anti-rat BP serum is inhibited by varying amounts of non-radiolabelled BP using polyethylene glycol to precipitate antibody-bound ^{125}I-BP[13,15] A similar radioimmunoassay kindly performed by Dr V. Lennon of the Salk Institute, showed that 1.0 mg of BSCP contained 15 ng of BP. Thus the 2.0 mg of BSCP required for protection against EAE could not have contained more than 30 ng of BP, an amount far less than the 10,000 ng of BP reported to be the lowest amount capable of preventing EAE in the guinea pig[16].

The mechanism of the protective action of SCP is not clear. Data on the effect of SCP-pretreatment on the lymphocytes mediating EAE suggest that the mechanism is likely to be complex For example, SCP-pretreated guinea pigs regularly displayed delayed skin hypersensitivity to BP after challenge but there was no correlation between the degrees of cutaneous reactivity and protection from disease[9].

The protective activity may be related to the location of SCP in nervous tissue. Indirect immunofluorescence studies using rabbit anti-sera showed that SCP is localised in the axons of neurones in the central and peripheral nervous systems and seems to be absent from the surrounding myelin sheaths (unpublished results). Protective activity might also involve non-specific suppression of selected T-cell function by anti-SCP antibody in a manner analogous to that of the anti-θ thymic antibody because RSCP is a component of rat thymus[10]

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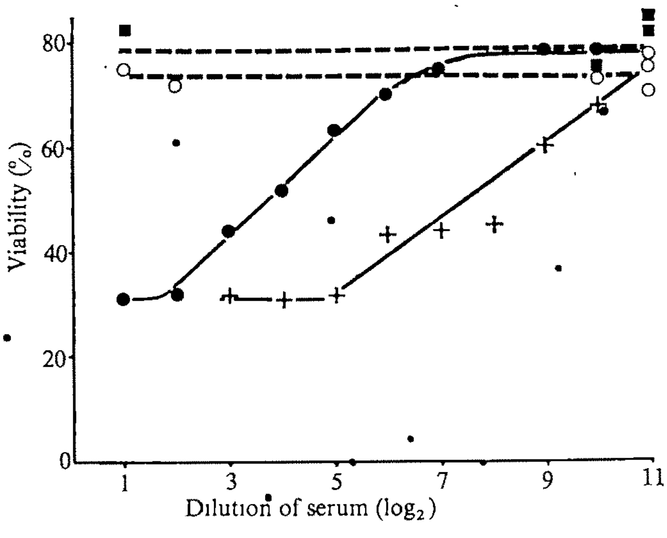
Gangliosides as surface antigens on cells isolated from the rat cerebellar cortex

GANGLIOSIDES are a class of complex glycolipids characterised by their content of *N*-acetyl neuraminic acid. They are present in many tissues, but are found in highest concentration in those of the nervous system. At one time they were thought to be characteristic of the neurone, but the analysis of cell fractions from a number of species indicates that they are present in glia and myelin as well as in the neuronal elements[1,2]. The investigation of subcellular fractions suggests that most of the ganglioside is located in the plasma membrane and their functional significance is still uncertain. We have investigated the effect of anti-ganglioside serum on suspensions of cerebellar cells from immature rats. Trypan blue exclusion and ^{51}Cr release methods were used as indicators of cytotoxic activity The results indicate that ganglioside is located at the outer surface of the plasma membrane in 60–80% of the cells. The remainder do not seem to exhibit the reactive hapten upon their surface. The experiments also demonstrate the value of complement mediated cytotoxicity as a method of studying surface lipid haptens in the cells of the nervous system.

Suspensions of cerebellar cells were freshly prepared from rats 5–12 d post-natally[3]. The final suspension contained 5–15 × 10^6 cells per ml in Krebs bicarbonate ringer containing glucose, 15 mM, and bovine serum albumin, 0.3% w/v Antisera were raised in rabbits against the total ganglioside fraction isolated from postmortem human brain tissue The total ganglioside fraction was isolated from the chloroform–methanol extract by partitioning against saline, and further purified from the upper phase by dialysis and barium precipitation by the method of Wolfe[4]. Rabbits were

Fig. 1 Cytotoxicity of rabbit anti-ganglioside serum for dissociated rat cerebellar cells. ●, Anti-ganglioside serum/cerebellar cells from 6-d-old rats, +, Anti-ganglioside serum/cerebellar cells from 12-d-old rats, ■, control normal rabbit serum/cerebellar cells from 6-d-old rats, ○, control. normal rabbit serum/cerebellar cells from 12-d-old rats Guinea pig complement (25 μl) was added followed by a further 0.5-h incubation Cytotoxicity assays using Trypan blue (0.2%) were carried out in micrtoture trays. The cell suspension was diluted to contain 10^6 cells ml^{-1} Antiserum (25 μl) was added to 50 μl cell suspension and incubated at 37 °C for 30 min Fresh neat guinea pig serum (25 μl) containing complement was then added and after shaking, incubated for a further 30 min. For counting, the supernatant was removed and one drop of Trypan blue (0.2%) in Krebs ringer added



immunised with the total ganglioside mixture and either bovine or human serum albumin as carrier, emulsified in complete Freunds adjuvant[5]

The viability of the cell preparation was generally better than 90% (mode of 96%, range 80–99%). Exposure to normal rabbit serum and guinea pig complement did not affect the viability over a period of hours Anti-ganglioside serum was found to be cytotoxic for cerebellar cells in the presence of guinea pig complement (Fig. 1). A fraction of the cells, however, were found to be resistant, and even repeated exposure to antiserum and complement failed to increase the proportion of the cells killed (Fig 2). From Fig 1 it can be seen that antiserum was more effective against cells from 10-d-old animals than those from 5-d-old animals, although the proportion of cells killed remains approximately constant. The total ganglioside concentration of the rat cerebellum increases sharply 5 d after birth, indicating an increase in membrane concentration[6]. The present findings suggest that this increase may initially be restricted to certain cells, at least for the gangliosides GM1 and GD1b, the suspected antigenic members of the ganglioside mixture[7,8].

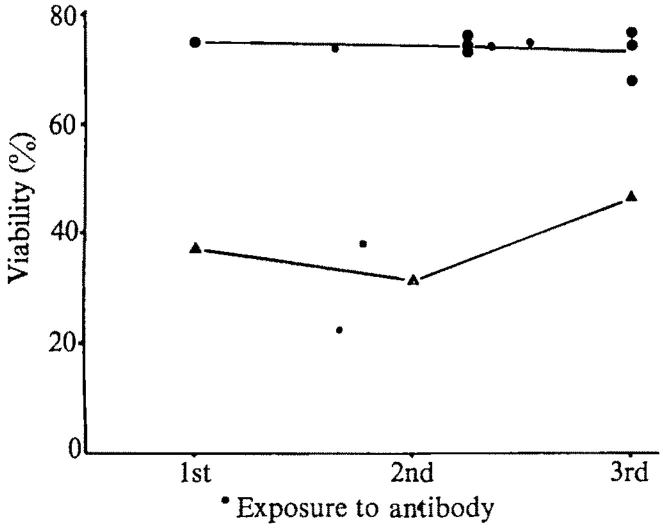


Fig. 2 Effect of repeated exposure of cerebellar cells to anti-ganglioside serum and complement. Viability declines to 30–40% on addition of antibody and reaches a plateau The surviving cells are unaffected by subsequent treatment with antibody ▲, Anti-ganglioside serum, ●, normal rabbit serum (control)

The specificity of the antibody for ganglioside was demonstrated by absorption with a hapten–lecithin mixture in the weight ratio of 1:3. Ganglioside (0.01 mg) completely removed all cytotoxic activity from 0.1 ml serum Lecithin alone was ineffective Absorption with an acetone dried rat brain powder removed both complement-fixing activity against ganglioside and cytotoxicity to cerebellar cells. The activity of the antiserum was unaffected by similar absorptions with rat liver powder A further indication of the specificity of the reaction for ganglioside is that anti-galactocerebroside sera having high titres of complement-fixing activity against galactocerebroside–auxiliary lipid mixtures and purified myelin fractions of rat brain, was not cytotoxic for cerebellar cells.

The antigen on the cell surface seems to be a glycolipid and not a glycoprotein. After partitioning of a chloroform–methanol extract of cerebellar cells the ability to absorb out cytotoxic acivity from antiganglioside serum was present in the water-soluble upper phase The lower phase lipids and the insoluble residue were ineffective (Fig 3). Absorption of antibody was dependent on the presence of the auxiliary lipid lecithin, further confirmation that we are dealing with a lipid hapten.

Ganglioside can be demonstrated in cells of the granular layer of the cerebellum of the adult rat by the immuno-peroxidase method[9] Histochemical localisation in tissue sections, however, does not provide information on the number of cells with surface antigen The present data suggests that in the immature animal, at least, this is in the region of 60–80%. The nature of the cell fraction

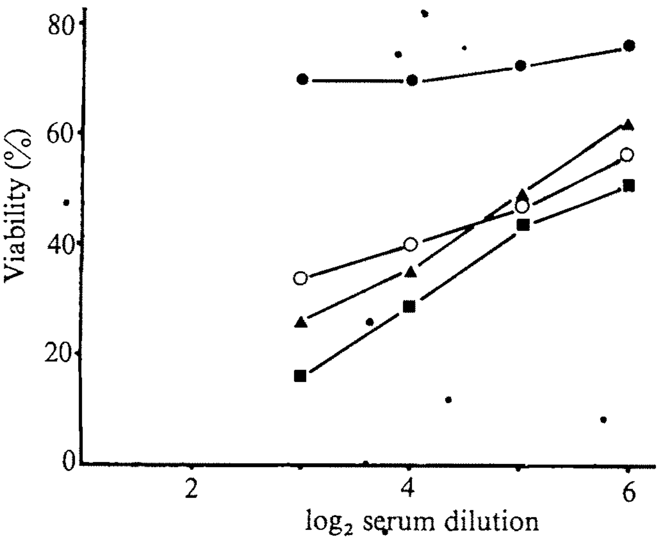


Fig. 3 Cytotoxicity of anti-ganglioside serum with fractions obtained by chloroform–methanol treatment of cerebellar cells. ●, Upper phase absorbed; ○, residue absorbed, ▲, lower phase absorbed, ■, unabsorbed serum About 5 × 10^7 cerebellar cells ml^{-1} were extracted in chloroform–methanol (1 1, v/v) This insoluble residue was centrifuged and resuspended by homogenisation and ultrasonication in 0 5 ml saline Chloroform was added to the lipid extract to make it 2:1 chloroform–methanol and then partitioned against 0 2 volume of saline Both upper and lower phases were evaporated to dryness under N_2 Lecithin (100 μg) was added to the upper phase Both residues were re-suspended in 0 5 ml saline with the aid of ultrasound One-quarter dilution of serum (0 5 ml) was added to each of the three fractions and stored at 4 °C overnight After centrifugation the cytotoxic activity of the absorbed and a control serum for rat cerebellar cells was determined Cytotoxicity was measured by ^{51}Cr release Cerebellar cells were incubated with 50 μCi ^{51}Cr in saline for 0 5 h They were diluted in 3 ml Krebs buffer and centrifuged through 4% bovine serum albumin in Krebs buffer to obtain labelled intact cells The supernatant was removed and the walls of the tube wiped before resuspending. Cell suspension (100 μl), serum (50 μl) and guinea pig complement (50 μl) were incubated for 1 h at 37 °C The reaction was stopped in an ice bath and 1 0 ml ice-cold medium added. After centrifugation 0 6 ml supernatant was removed and the radioactivity in supernatant and infranatant counted

$$\%\text{Viability} = \frac{\%\,\text{Cr release (serum + complement} - \%\,\text{Cr release (complement alone)}}{\%\,\text{Cr release (in water)} - \%\,\text{Cr release (complement alone)}}$$

lacking surface ganglioside is unknown It may constitute a particular cell type or merely reflect the immaturity of a proportion of cerebellar cells at this stage of development The maximal rate of ganglioside increase in the developing rat brain occurs 10–20 d postnatally, although increased synthesis can be detected earlier—before the onset of myelination. The enzyme glucosyl transferase, which is thought to be involved in ganglioside synthesis, reaches maximal activity by day 7 (ref. 10).

The demonstration that at least some of the ganglioside in brain cells is accessible to antibody at the cell surface suggests that immunohistochemical methods may profitably be applied to localise these haptens on the cell membrane It also raises the possibility that gangliosides may furnish a range of cell-surface markers for the identification and separation of cell types in the central nervous system

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Ciliary dyskinesia factors in cystic fibrosis and asthma

CYSTIC fibrosis (CF) homozygotes and heterozygotes carriers for CF harbour a factor in their serum (termed CDF) which causes ciliary dyskinesia when applied to ciliated epithelial tissue derived from rabbit trachea[1–4] Spock *et al.*[1] reported that serum ciliary dyskinesia activity (CDA) was a specific marker for the CF gene Conover *et al.*[5] have reported that a CDA is also found in sera from individuals with various respiratory and autoimmune disorders; they have since suggested that the CF-ciliary dyskinesia factor was C3a (anaphylatoxin), and proposed that a defect or deficiency in the anaphylatoxin inactivator was the primary gene defect in cystic fibrosis[6,7] Other laboratories have been unable to detect CF-CDF using a rabbit tracheal bioassay for the detection of the CF-CDF (refs 8–10) Using a modified rabbit tracheal bioassay, we have confirmed that serum from individuals with bronchial asthma and serum from homozygotes and heterozygotes for CF cause ciliary dyskinesia, whereas normal healthy control sera do not. Most individuals with asthma, however, were also found to have serum which causes a subsequent ciliostasis All sera tested by bioassay for CFP by electrofocusing indicated that all CDA-positive sera except sera from seven of eight individuals with asthma were also positive for cystic fibrosis protein (CFP) The CFP is a small (molecular weight 5,000–9,000), positively charged compound and is found in sera from most homozygotes and heterozygotes for CF (refs 11–16). These two findings suggested that the CDAs in CF sera and in asthmatic sera could be due to two different substances. We have developed two simple chromatographic techniques which can separate CDAs in CF and asthmatic sera. Our results indicate that they may be useful in purifying a CF-specific CDA Purification of this CF-CDA is a prerequisite for its biochemical characterisation and for elucidating its role in the pathophysiology of CF (ref 17).

Our rabbit tracheal bioassay[4] involved tissue selection according to the criteria of Conover *et al*[2], and bioassay following the protocol of Spock *et al*[1] with minor modifications The assay can reliably detect a ciliary dyskinesia activity (CDA) in sera from both heterozygotes and homozygotes for CF without fractionation or concentration of the serum[1,4]. Unlike the assay of Conover *et al*[2], all sera can be screened at 27 °C. Prewarming the serum to 37 °C before bioassay increased the kinetics of the reaction sufficiently to allow the determination of CDA-positive individuals in 35 min or less. Using our methods, 16 CF homozygote sera reacted in 14±8 min (mean±s d.) and 15 OHCF sera required 25±8 min, whereas 13 of 14 normal healthy control sera failed to react by 60 min (the end of the assay period[4]) Our results using whole serum for analysis confirm those of Spock *et al*[1] and Conover *et al.*[2] in that sera from normal healthy donors does not elicit a ciliary dyskinesia reaction, whereas both homozygotes and heterozygotes for CF apparently do harbour a CDA.

To evaluate the specificity of the CDA detected, we bioassayed serum from eight patients with bronchial asthma[4,18] In agreement with Conover *et al.*, we found that all eight asthmatic sera caused a ciliary dyskinesia reaction; however, sera from asthmatics generally caused a subsequent ciliostasis (cessation of ciliary beating) Concurrent evaluation of all sera for CDA by bioassay and for CFP by electrofocusing showed that all CDA-positive sera except seven of the eight asthmatic sera were also positive for CFP (our specific marker for the CF gene)

These two findings indicated that the activity detected in patients with asthma and other respiratory diseases[5] could be a substance different from a CF-specific CDF To test our hypothesis we set out to purify the CDAs in asthmatic, CF homozygote and OHCF sera We found two simple chromatographic procedures for separating and therefore distinguishing between the CDA found in CF and asthmatic sera The first procedure involves fractionating whole serum on DEAE-cellulose (DE-52, Whatman) using 0 005 M Tris-HCl buffer, *p*H 8 6, and a two-step NaCl gradient (0.00 M NaCl, 0 05 M NaCl) for elution. The second procedure involves fractionation of whole serum by Sephadex G-200 (Pharmacia) gel filtration, using 0.01 M phosphate, 0 15 M NaCl buffer, *p*H 8 0, for elution[18]

For DEAE–cellulose chromatography, 2.0 ml of serum is dialysed overnight at 4 °C in Spectrapor 3 dialysis membrane tubing (Spectrum Medical). Spectapor 3 tubing has a known molecular weight retention of 3,500 and will effectively retain CFP, CF-CDF, and C3a if free in solution[7,12]. The serum is then applied to a column pre-equilibrated with eluting buffer. An initial IgG-containing fraction (Fraction DEAE-I) is obtained by elution with Tris-HCl without NaCl This fraction (DEAE-I) contains only the most cathodally migrating gamma-globulin fraction of serum [isoelectric point (*p*I) 8–9 (refs 18, 19)] The column is then eluted with Tris-HCl plus 0.05 M NaCl, and a second IgG-containing fraction (plus contaminants) is eluted (DEAE-II). Both DEAE fractions are lyophilised, reconstituted to 2 0 ml, and dialysed against PBS (0 01 M phosphate, 0 015 M NaCl, *p*H 7 2) in preparation for bioassay

For Sephadex G-200 chromatography, 2.0 ml of serum is fractionated and the effluent is separated into six fractions (Fig. 1) Each fraction is then prepared for bioassay as described for DEAE-I and II

Representative results (Table 1) clearly indicate that the asthmatic CDA is found in DEAE-II and Sephadex G-200 Fraction II, whereas the CDA found in CF and OHCF sera elutes in DEAE-I and Sephadex G-200 Fractions III and IV (weak CDA) Our CFP detected by electrofocusing is also found in DEAE-I and Sephadex G-200 Fraction III[12,18,19]. Fractionation of serum from a heterozygote carrier (CFP positive) with bronchial asthma confirmed the existence of two distinct activities in asthmatic and CF sera, as this serum had activity in DEAE-I and II and in Sephadex G-200 Fractions II, III and IV (Table 1).

We conclude that the CDAs in asthma and CF are distinct substances. Our findings may facilitate purification of what we feel is a CF-specific CDF from CF and OHCF serum Purification is a prerequisite to elucidating the exact nature of this substance and its role in the pathophysiology of CF.

Most recently, we have found that an 850-fold purified preparation of the CDF in asthmatic serum reacts with antisera to human C3a, whereas a 650-fold purified active preparation of CF-CDF (at the same protein concentration) does not[18,19] We feel the different reactivities with anti-C3a antiserum further distinguish the asthmatic and cystic CDAs

Of related interest, are the results of Bowman *et al.*[20,21] concerning the specificity and identification of a ciliotoxic factor (CTF) found in serum from homozygotes and heterozygotes for

Fig. 1 Fractionation of 2 0-ml samples of human sera by gel filtration on Sephadex G-200 Fractions (2 0 ml) were collected at a flow rate of 5 ml h^{-1}. V_0, V_T void volume and total column bed volume HSA human sera albumin

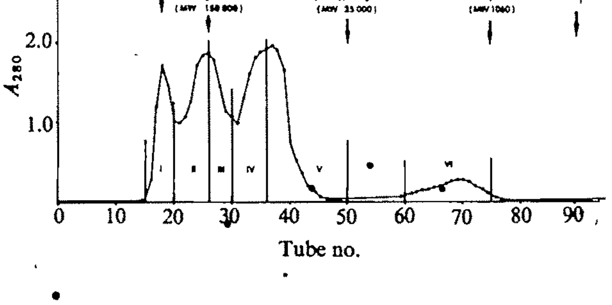

Table 1 Separation of ciliary dyskinesia activity by chromatography

Serum	DEAE–cellulose fraction I	DEAE–cellulose fraction II	Sephadex G-200 fraction I	II	III	IV	V	VI
Normal	—	—	—	—	—	—	—	—
CF homozygote	CDA*	—	—	—	CDA	CDA	—	—
Obligate heterozygote carrier	CDA	—	—	—	CDA	CDA	—	—
Asthmatic	—	CDA	—	CDA	—	—	—	—
Asthmatic-heterozygote carrier	CDA	CDA	—	CDA	CDA	CDA	—	—

*CDA fraction produced ciliary dyskinesia by 35 min

CF. This factor is now termed 'cystic fibrosis mucociliary inhibitor' (CFMI) CTF is detected using an oyster gill ciliary bioassay[22] The investigators claim that CTF is specific for the CF gene since sera from asthmatic, allergic and normal healthy control subjects do not harbour a CTF. Although this CTF has a molecular weight, cationic charge and other properties which resemble those described for the CDF of Conover *et al* [6,7], Barnett and Bowman[21] claim that the CTF is not C3a either.

Although the identity of CTF and the CF-specific CDF described here are unknown, neither our studies nor those of Bowman and co-workers[20,21], lend support results[6,7] which suggest that the CF-CDF is C3a. It remains possible, however, that the elusive 'CF factor(s)' could be related to some other component found in the complement, kinin[23], or fibrinolytic systems.

They could be, for example, abnormal fragments of C3a, C5a or other active agents which affect membrane permeability or ion transport (that is, polyamines or their aminoaldehyde derivatives [24–26]) We have recently shown that α_2 macroglobulin (α_2M) is abnormal in CF and have hypothesised that this abnormality can explain the presence of 'CF Factors' (whatever their exact biochemical nature may be) in various body fluids of CF patients due to defective regulation of serum and cellular derived proteases by CFα_2M In addition, a defective α_2M (possibly coupled with an abnormality in polyamine metabolism) may be responsible for the general pathophysiology of this disease[24–26] Our hypothesis and findings concerning α_2M have recently been confirmed by Shapira *et al.*[27]

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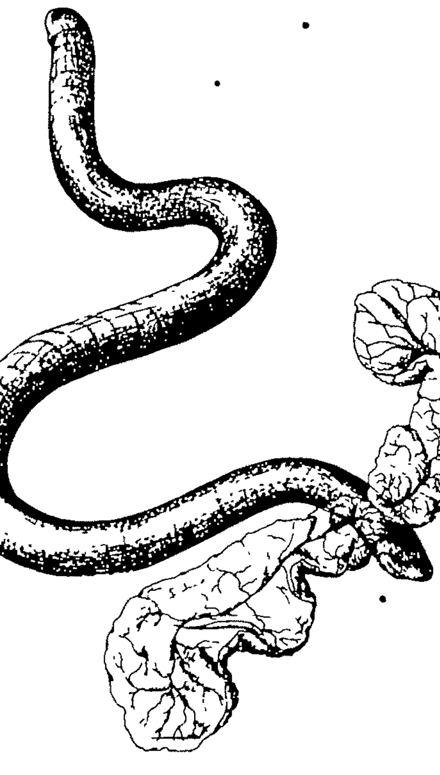
Blood respiratory properties of a viviparous amphibian

BURROWING apodan amphibians (order Gymnophiona) are relatively common in the Amazon River Basin of South America but the physiology of the group has been little studied. The animals are usually fossorial, living burrowed in the mud banks of small tributaries of the main river. The burrows, which they presumably dig, are small and conditions are often both hypoxic and hypercarbic. Animals 0.25–0.75 m long and weighing 100–600 g, found near the air–water interface, were dug out of the mud banks and immediately taken to the laboratory of the research vessel RV Alpha Helix for study. Several species of burrowing apodans occur in the area, but the most common is *Typhlonectes compressicauda*, the animal used in this study. We report here on the blood respiratory properties of this organism.

Most female *T compressicauda* captured had uteri containing several gilled larvae. The gills are large well vascularised jelly-like structures (Fig 1) and the gill surfaces make close contact with the internal lining of the uterus. The structural relationship between larvae and parent in *Typhlonectes* clearly suggested a maternal supply of oxygen, and oxygen equilibrium curves were therefore constructed using pooled blood from three pregnant adult females and 27 larvae removed from several animals. Whole blood was

Fig. 1 Gilled larva removed from the uterus of a female *Typhlonectes compressicauda*, a viviparous amphibian.



used in all determinations and the technique of Edwards and Martin[1] in curve construction was used

Adult blood at a P_{CO_2} of 11.4 torr showed a sigmoid oxygen equilibrium curve with a P_{50} of 22 2 torr (Fig. 2). Increasing the P_{CO_2} to 17.1 torr increased the P_{50} slightly to 22.6 torr, indicating no appreciable Bohr shift. The absence of a Bohr effect would be physiologically advantageous to these animals living in small mud burrows in which the ambient P_{CO_2} fluctuates extensively A Bohr effect in these conditions might result in the undesirable situation of facilitating oxygen unloading at the skin.

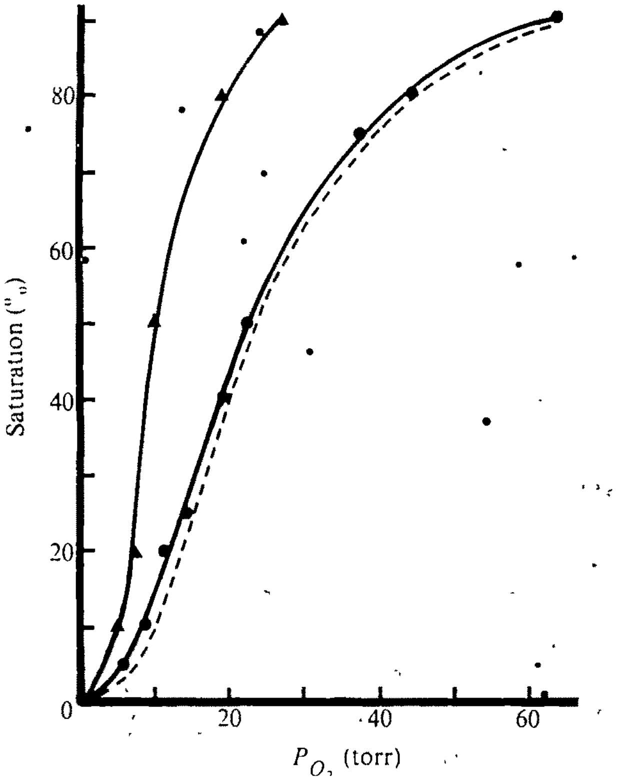


Fig. 2 Oxygen equilibrium curves of *T compressicauda*. ▲, Foetal curve from gilled larvae removed from uterus P_{CO_2} 11 4 torr.) ●, Maternal curve (P_{CO_2} 11.4 torr) Dashed line, maternal curve (P_{CO_2} 17.1 torr).

The foetal blood had an O_2 equilibrium shifted markedly to the left of the maternal blood (P_{50} 9.3, P_{CO_2} 11 4 torr) This intriguing feature would assist in maternal–foetal gas exchange across the uterine–gill membranes because high haemoglobin saturation of foetal blood is achieved with only moderate saturation levels of maternal blood.

Foetal–maternal shifts have been described in the viviparous garter snake *Thamnophis*[2], the ovoviviparous dogfish *Squalus*[1] and the oviparous teleost *Scorpaenichthys*[3], while Riggs[4] has shown bullfrog tadpole curves shifted to the left of the adult. We believe our report to be the first description of a foetal–maternal shift in the Amphibia

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One-to-one binding of a purified scorpion toxin to Na channels

DETAILED elucidation of the molecular organisation which controls ionic flow through the excitable membranes has been prevented by a difficulty in isolating substances to which characteristic features of the ionic channels can be attributed[1–3]. In the study of the cholinergic receptor, polypeptide α-toxins from certain snakes have been successfully used for the identification and purification of nicotinic receptors[4] Thus, agents analogous to snake toxins may be of a decisive value in the molecular approach to ionic channels, and toxins from certain scorpions are among the most promising candidates[5–8]. Scorpion toxins seem to act by modifying kinetic properties of Na channels as well as by suppressing the current through K channels[9–11] But, no quantitative analysis was made on an electrophysiological basis to show the precise nature of the toxin-binding We describe here a study of the effects of a toxin from *Leiurus quinquestriatus* on Na, Ca and K currents in the tunicate egg membrane, where each ionic current proved to be essentially identical with that in other excitable membranes[12,13] The discrete critical membrane potentials for the activation of these currents in the egg facilitate discrimination of the respective currents only by adjusting potential steps in the voltage-clamp condition Thus, quantitative aspects of the inactivation kinetics of Na current can be analysed conveniently in this preparation

A crude toxin (Sigma) induced a slowly inactivating component of the Na current and enhanced it in a dose-dependent manner (Fig. 1, inset). The effective component was therefore fractionated on a CM-Sephadex C-25 column, taking the amount of the current remaining at a definite time after the potential step as a measure of toxic activity Since the action of the toxin on the egg membrane was highly reversible, all recordings for the respective fractions were made with the same single preparation Active fractions were collected, rechromatographed and finally gel-filtered on Sephadex G-50 There was substantial agreement between the toxic activity and absorbance profiles during the last filtration procedure SDS-8 M urea gel electrophoresis[14] of the isolated toxin showed a purity of >90%. Molecular weight was estimated to be 6,000 by gel-filtration on a Sephadex G-50 column

The effect of varying concentration of this purified toxin on the Na current is illustrated in Fig 2*a* In the presence of the toxin, the current was inactivated in two phases—an initial fast phase (dotted line) and later slow phase (solid line) It is essential to note that an increase in the dose enhances the slow component with a unique time constant of 309 ± 6 ms (about 40-fold greater than toxin-free control) The slow components were subtracted from the respective original current traces and the fast components isolated analysed (Fig 2*b*), the fast components had a common inactivation time constant of 8 3 ± 0 3 ms regardless of the dosage The value was comparable with 8 5 ms obtained for the toxin-free control The amount of this fast component was decreased as the final concentration of the toxin in the medium was increased It was strongly suggested that the slow component is the current through Na channels modified by the binding of the toxin, while the fast one is that through intact Na channels To analyse the dose–response profile for the toxin, the slow component corrected to the initial zero time of the potential step (I_0^* in Fig 2*a*) was illustrated in a double reciprocal plot (Fig. 2*c*). A straight line was obtained, yielding K_D value of 0.46 μM. When the reciprocal of the fast component similarly corrected to zero time (I_0 in Fig 2*b*) was plotted directly against toxin concentration, a straight line was again obtained (Fig 2*d*) The extrapolated value of the I_0 to toxin-free condition (intercept on the ordinate) was in precise agreement with the peak value of the toxin-free control current, while from the intercept on the abscissa the same K_D value of 0 46 μM was deduced as in the case of the slow component Thus purified scorpion toxin binds to Na channel in one-to-one stoichiometry and modifies its inactivation kinetics. The maximum I_0^* was

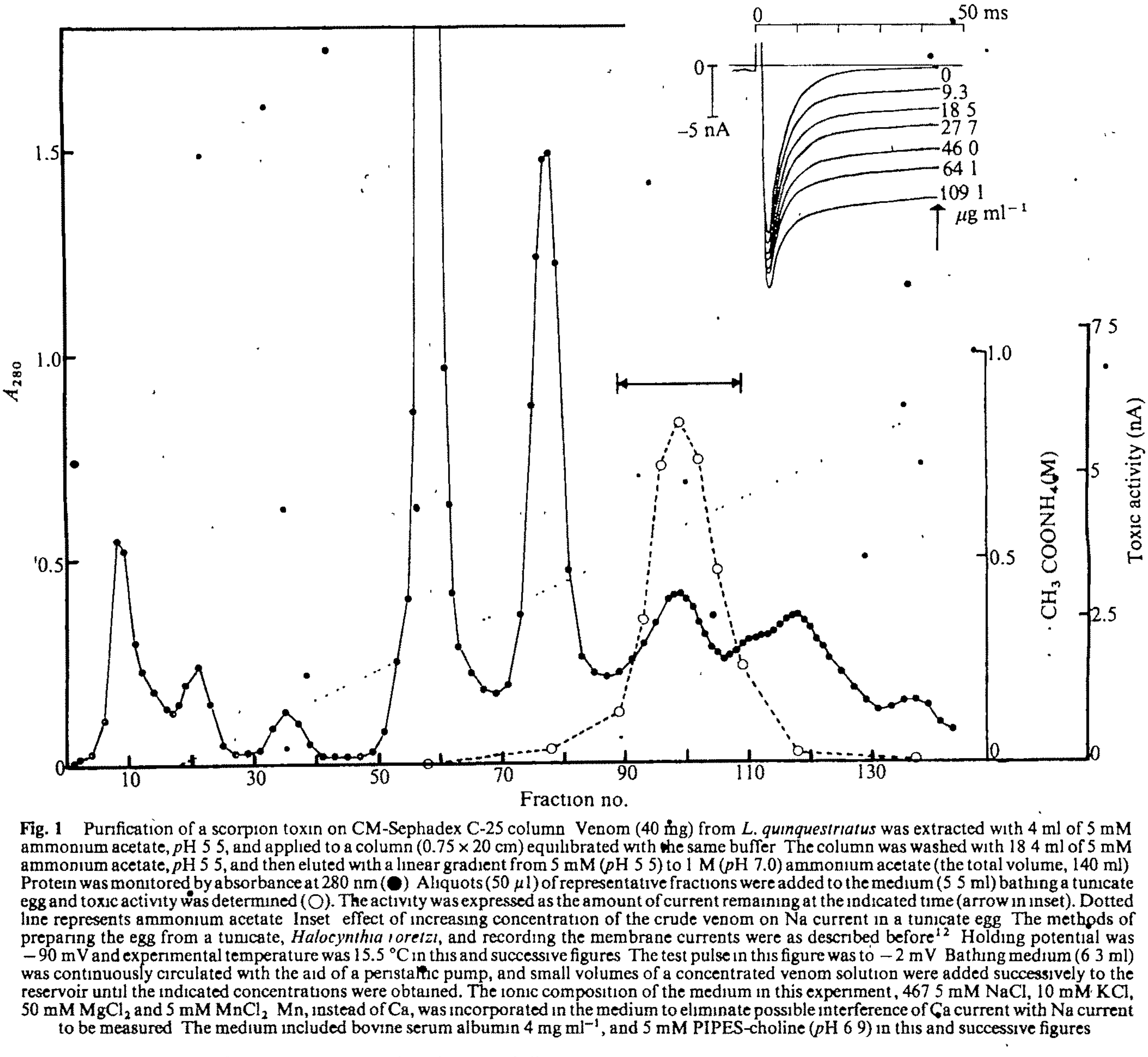

Fig. 1 Purification of a scorpion toxin on CM-Sephadex C-25 column Venom (40 mg) from *L. quinquestriatus* was extracted with 4 ml of 5 mM ammonium acetate, *p*H 5 5, and applied to a column (0.75 × 20 cm) equilibrated with the same buffer The column was washed with 18 4 ml of 5 mM ammonium acetate, *p*H 5 5, and then eluted with a linear gradient from 5 mM (*p*H 5 5) to 1 M (*p*H 7.0) ammonium acetate (the total volume, 140 ml) Protein was monitored by absorbance at 280 nm (●) Aliquots (50 μl) of representative fractions were added to the medium (5 5 ml) bathing a tunicate egg and toxic activity was determined (○). The activity was expressed as the amount of current remaining at the indicated time (arrow in inset). Dotted line represents ammonium acetate Inset effect of increasing concentration of the crude venom on Na current in a tunicate egg The methods of preparing the egg from a tunicate, *Halocynthia roretzi*, and recording the membrane currents were as described before[12] Holding potential was −90 mV and experimental temperature was 15.5 °C in this and successive figures The test pulse in this figure was to −2 mV Bathing medium (6 3 ml) was continuously circulated with the aid of a peristaltic pump, and small volumes of a concentrated venom solution were added successively to the reservoir until the indicated concentrations were obtained. The ionic composition of the medium in this experiment, 467 5 mM NaCl, 10 mM KCl, 50 mM $MgCl_2$ and 5 mM $MnCl_2$ Mn, instead of Ca, was incorporated in the medium to eliminate possible interference of Ca current with Na current to be measured The medium included bovine serum albumin 4 mg ml⁻¹, and 5 mM PIPES-choline (*p*H 6 9) in this and successive figures

slightly lower than that of I_0, suggesting a reduced permeability coefficient for the toxin-modified channels (about 85% of the control).

Figure 3 shows the channel specificity in the action of the purified toxin. Li current through Na channels[12] was modified as above (Fig 3*a*), but *K* and Ca channels were not affected (Figs 3*b*, *c*). Further, a detailed analysis indicated that the toxin exerts little, if any, influence on the activation kinetics of Na channels

Reduction of the cationic concentration in the medium led to a decrease in the K_D value of the toxin-binding When the ratio of unmodified (I_0) to modified (I_0^*) currents at varying external Na concentrations and at a constant level of toxin was plotted against $[Na]^2$, a linear relation was obtained (Fig. 2*e*). Since this ratio is expected to be proportional to the K_D value in case of one-to-one binding, the competitive inhibition of the toxin-binding by Na ions was indicated Essentially similar results were obtained with Li, Ca, Mn and La ions in the medium. This suggests that there are multiple binding sites for cations, the cooperativity of these sites may differ to a certain extent depending upon the ionic species to be bound, because Hill coefficients varied around 2 according to the species of cations tested.

Our results clearly demonstrate, on an electrophysiological basis, that a toxin purified from venom of *L. quinquestriatus* specifically binds to Na channels in the tunicate egg membrane and modifies almost exclusively the inactivation kinetics. Electrically excitable neuroblastoma cells have been shown to contain a similar

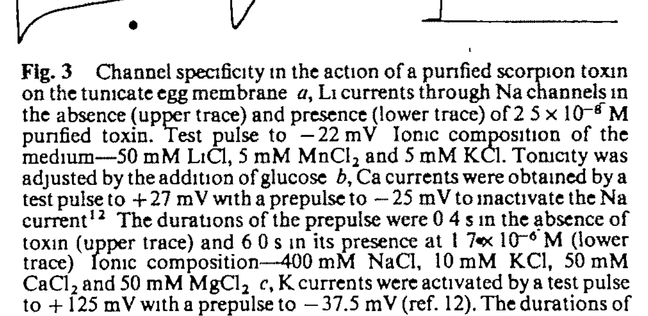

Fig. 3 Channel specificity in the action of a purified scorpion toxin on the tunicate egg membrane *a*, Li currents through Na channels in the absence (upper trace) and presence (lower trace) of 2 5 × 10^{-8} M purified toxin. Test pulse to −22 mV Ionic composition of the medium—50 mM LiCl, 5 mM $MnCl_2$ and 5 mM KCl. Tonicity was adjusted by the addition of glucose *b*, Ca currents were obtained by a test pulse to +27 mV with a prepulse to −25 mV to inactivate the Na current[12] The durations of the prepulse were 0 4 s in the absence of toxin (upper trace) and 6 0 s in its presence at 1 7 × 10^{-6} M (lower trace) Ionic composition—400 mM NaCl, 10 mM KCl, 50 mM $CaCl_2$ and 50 mM $MgCl_2$ *c*, K currents were activated by a test pulse to +125 mV with a prepulse to −37.5 mV (ref. 12). The durations of the prepulse were 0.4 s in the absence of toxin (upper trace) and 10 s in its presnce at 0.47 × 10^{-6} M (lower trace) Ionic composition was the same as in Fig 1 In *b* and *c*, it was confirmed that the toxin effectively modified the inactivation kinetics of Na current.

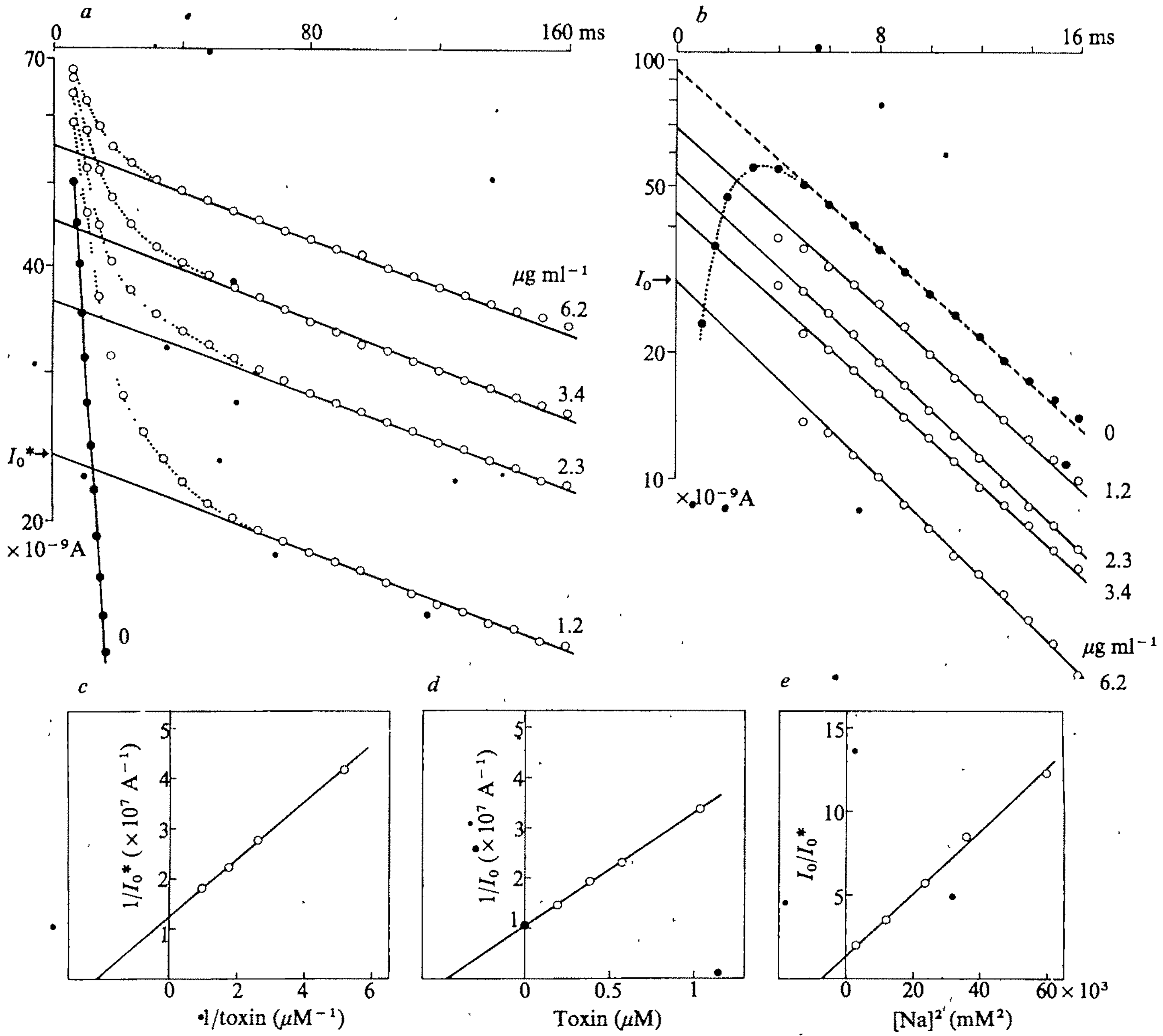

Fig. 2 Effect of a purified toxin on inactivation process of the Na current *a*, Na currents were measured as described for Fig 1, except for the test pulse to −9 5 mV Purified toxin was added successively until the indicated concentration was obtained The logarithm of the current in the presence (○) or absence (●) of the toxin was plotted against time *b*, The slow components (solid lines in *a*) were subtracted from the respective original currents and the logarithms of the fast components isolated were plotted against time (○) For a comparison, the Na current in toxin-free medium was also included (●) *c* and *d*, the reciprocals of the slow and fast components corrected to zero time (I_0^* in (*a*) and I_0 in (*b*)) were plotted against the reciprocal of toxin concentration (*c*), and against toxin concentration (*d*), respectively *e*, Inhibition of toxin-binding by Na ions External Na concentration was successively increased at a constant level of the toxin ($1\ 7 \times 10^{-8}$ M) and with 5 mM $MnCl_2$ and 5 mM KCl $MgCl_2$ was omitted and tonicity was adjusted by the addition of glucose The ratio of I_0 to I_0^* at the indicated concentration of Na was calculated as in (*a*) and (*b*), and was plotted against $[\text{Na}]^2$. The Na currents used for this estimation were obtained with a test pulse to −20 mV

single class of binding sites for the toxin from *L quinquestriatus*[7]. In this experiment the active toxin had been isolated by monitoring its stimulative effect on the tetrodotoxin-sensitive ^{22}Na influx induced by the simultaneous presence of veratridine, whereas our purification procedure was based directly on the electrophysiological effect of the toxin Although the purified toxin may be identical in these two experiments, the reported K_D values differ widely (1.3–2 4 nM in neuroblastoma and 460 nM in the tunicate egg) This apparent discrepancy, however, can largely be attributed to a higher cationic concentration in the artificial sea water used in our experiment. In fact, the K_D of about 10 nM was estimated in the tunicate egg in a cation-free condition, taking the competitive inhibition by cations in the medium into consideration. It is likely that the presumptive inactivation particles of Na channels[15] bear multiple sites that bind various cations, and these sites may be blocked by a single scorpion toxin molecule to result in the retardation of the inactivation gating.

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Does intracellular sodium modify membrane permeability to sodium ions?

IN sodium transporting epithelia the asymmetrical cells have the apical face specialised for sodium entry while the basolateral membranes are concerned with sodium export, the accompanying anion passing passively. Epithelia exposed to various sodium concentrations may need to control sodium entry as a way of regulating the intracellular concentration. Some amphibian epithelia can show adaptive changes in their transporting capacity with changes in the ambient sodium concentration[1–3]. In addition, rapid changes in permeability of the apical face occur with time constants of a few seconds when epithelia are exposed to step changes in sodium concentration[4]. While the slow adaptive changes may involve alterations in synthesis of membrane macromolecules important for membrane permeability, the rapid changes are likely to result from fast changes involving the existing membrane apparatus. One effector agent for the rapid reactions may be the intracellular sodium concentration and the experiments reported here were designed to test this hypothesis. We have manipulated frog skin epithelium in ways to increase the intracellular sodium content and found that the density of sodium channels in the apical face, measured by labelling with ^{14}C-amiloride, is reduced in these circumstances.

^{14}C-amiloride has been used previously to label sodium channels in frog skin and isolated toad bladder cells[3,5–7], and the amount of ^{14}C-amiloride displaced from binding sites by unlabelled drug when the former is used at a concentration of 10 nM in the presence of 1.1 mM Na^+ is a measure of binding to sodium channels. As external sodium is reduced the apparent affinity of amiloride increases[8]. Labelling is carried out with only low concentrations of sodium present to minimise the concentration of ligand required, and so reduce nonspecific binding.

Part of the evidence that amiloride binds at, or close to, sodium entry sites is given by the correlation of site density and the level of sodium transport which can be maintained by the tissue. Epithelia deprived of sodium for 1 week have been found to have a threefold increase in site density and can, when re-exposed to sodium, maintain a transport level three times that of control skins[3].

We measured ^{14}C-amiloride binding in the mucosal surface of frog abdominal skin (*Rana temporaria*, area 9.6 cm^2) as described before[3,5,6,8]. To increase sodium concentration on the inside of the apical membrane the serosal surface was treated with ouabain or K-free solution to reduce or abolish the activity of the sodium pumps. Throughout the experiments the transepithelial voltage was maintained at zero by an automatic voltage-clamp device.

In each experiment, five measurements were made of the amount of displaceable amiloride bound to the skin. Subsequently, a further five measurements were made after ouabain had been used to reduce transport to a low level (about 90% inhibition).

Results of experiments in which amiloride binding was measured in the presence of 1.1 mM Na^+ are given in Table 1. After transport had been inhibited by ouabain there was no significant difference in the amount of amiloride bound. In these experiments, and before ouabain inhibition, the intracellular sodium concentration was in a steady state, in which sodium influx through the mucosal face was equal to sodium efflux through the serosal face. After ouabain, it is unlikely that the intracellular sodium concentration increased as the mucosal concentration was only 1.1 mM, and furthermore, the favourable electrical gradient for sodium entry could only decrease after sodium pump inhibition. Sodium can enter and leave the cells only by the mucosal face after pump inhibition, as shown by studies with the electron probe[9].

If the mucosal face of the skin is flushed with high sodium solution (111 mM) and then returned to low sodium (1.1 mM) conditions before binding is measured, the increased intracellular sodium concentration is lowered rapidly, mainly by pumping through the serosal surface. After ouabain, however, this exit route will be blocked. Experiments such as those described in Table 1 were reapted except that each binding measurement was preceded by a high sodium flush. The results (Table 2) show that inhibition of transport with ouabain reduces amiloride binding to 36% of its control value. If the equivalence between amiloride-binding sites and sodium channels is accepted, the result after ouabain corresponds to a decrease in the mucosal sodium permeability, effected by a reduction in availability of channels. An ouabain-like effect was also obtained when potassium was omitted from the serosal bathing solution; this produced a 31% reduction in channel density.

So far, our results indicate that an increase in intracellular sodium (or possibly a decrease of intracellular potassium) reduces amiloride binding, and therefore the availability of sodium channels, in the mucosal face of the cells. This may not, however, be due to intracellular sodium changes *per se*, but to other effects, such as a potential change across the mucosal membrane. For example, changes in transepithelial potential, V_T, change the extent of amiloride binding[6]. This could be clarified if it were possible to control the potential across the mucosal membrane,

Table 1 Effect of ouabain on amiloride binding in low sodium solution

	Control	After ouabain (10^{-3} M)
Total binding (10 nM ^{14}C-amiloride)	1.44 ± 0.07	1.99 ± 0.05
Non-displaceable binding (10 nM ^{14}C-amiloride plus 1 μM unlabelled amiloride)	1.20 ± 0.07	1.76 ± 0.05
Displaceable binding	0.23 ± 0.03 ($P < 0.001$)	0.20 ± 0.04 ($P < 0.001$)
	Δ = 0.03 NS	

Amiloride binding is expressed as pmol per 9.6 cm^2. The concentration of sodium was 1.1 mM. Means and s.e. are from results for 15 determinations in three separate skins. The significance of the specific (displaceable) binding was estimated using a paired *t* test. The serosal surface was bathed in Ringer's solution (mM, NaCl, 111, $CaCl_2$, 1; KCl, 2, Tris, *p*H 7.6, 5 and glucose 11.0). The mucosal solution contained only 1.1 mM NaCl and no glucose. ^{14}C-amiloride had a specific activity of 54 Ci mol^{-1}. NS, not significant.

Table 2 Effect of ouabain on amiloride binding in low sodium solution following a high sodium flush

	Control	After ouabain (10^{-5} M)
Total binding (10 nM ^{14}C-amiloride)	1.72 ± 0.10	2.02 ± 0.07
Non-displaceable binding (10 nM ^{14}C-amiloride plus 1 μM unlabelled amiloride)	1.47 ± 0.09	1.93 ± 0.07
Displaceable binding	0.25 ± 0.03 ($P < 0.001$)	0.09 ± 0.04 ($P < 0.02$)
	Δ = 0.16 $P < 0.005$	

Amiloride binding is expressed as pmol per 9.6 cm^2. Concentrations of sodium were 1.1 mM (low) and 111 mM (high). Means and s.e. are from results for 20 determinations in four separate skins. Ringer's solution was used to flush the mucosal surface before returning to low sodium solution to measure binding.

V_m, rather than across the whole epithelium. To achieve this we have used the potassium depolarised preparation of Morel and Leblanc[10,11]. The serosal side of the skin was bathed in a potassium-rich solution so that the resistance and potential across the serosal border are reduced to low values[12]. In this situation the transepithelial resistance is located mainly at the mucosal membrane, presumably making it possible to clamp this membrane at, or close to, zero. K-depolarised skins are able to maintain low level transport, and are still sensitive to amiloride and ouabain[10,11].

Table 3 gives the results obtained with K-depolarised skins. Again, amiloride binding was measured in low sodium conditions immediately after a high sodium flush. After ouabain had been used to inhibit serosal efflux, the amount of binding was decreased by 64%. It seems, therefore, that in this situation the reduction in binding could not have been entirely due to potential changes, but was more likely due to increased intracellular sodium.

At this stage it was necessary to check that ouabain did not cause a change in affinity of amiloride for the binding site so as to lead to reduced binding. Apparent affinities for amiloride (reciprocal of concentrations producing 50% inhibition of transport) were determined in six control skins bathed on both sides with Ringer's solution. The mean value (520 nM) fell within the normal range. Ouabain was then used to reduce sodium transport to about 30% of its control value. The affinity was redetermined on the residual sodium currents. No significant difference in apparent affinity was found after ouabain. We concluded that the changes in binding caused by ouabain were not the result of changes in the affinity of the ligand for the binding sites.

Table 3 Effect of ouabain on amiloride binding in low sodium solution after a high sodium flush with the serosal surface bathed in high potassium solution

	Control	After ouabain (10^{-3} M)
Total binding (10 nM ^{14}C-amiloride)	1.35±0.06	1.73±0.05
Non-displaceable binding (10 nM ^{14}C-amiloride plus 1 μM unlabelled amiloride)	1.10±0.08	1.63±0.06
Displaceable binding	0.25±0.03 ($P<0.001$)	0.09±0.02 ($P<0.005$)
	$\Delta=0.16$ $P<0.005$	

Amiloride binding is expressed as pmol per 9.6 cm². Concentrations of sodium were 1.1 mM (low) and 111 mM (high). Means and s.e. are from results for ten determinations in two separate skins. The serosal bathing solution was Ringer's in which all the NaCl was replaced with KCl.

The mean value for specific amiloride binding in control conditions in the experiments reported here was 0.243 pmol per 9.6 cm². This corresponds to 153 binding sites per μm² at an occupancy of 30–40% with 10^{-8} M amiloride, or about 400 binding sites per μm² at 100% occupancy, a value comparable with those reported elsewhere[3,5–8].

Our results provide a direct demonstration that an increase in intracellular sodium is accompanied by a reduction in the availability of amiloride-binding sites, and presumably of sites through which sodium entry can occur. This finding provides a mechanism to explain why the measured sodium influx through the mucosal face is inhibited by ouabain[11,13] and shows that reduced influx is not simply a result of a reducing gradient as the transport pool fills up. When both sodium and amiloride are present in the external solution they show competition, perhaps at a site which needs to be occupied for sodium ions to be translocated through the channel. Intracellular sodium seems to make a fraction of the channels unavailable to external Na$^+$ or amiloride, thus reducing the transepithelial current, possibly by interaction at a regulator site different from the site for translocation. The slow time course of the effect of step changes in external sodium concentration[4] might indicate that this too affects transport by increasing intracellular sodium.

The simple feedback operated by intracellular sodium may be an important homeostatic device for the transporting cell. For example, with conditions where the supply of energy for the pump is impaired, the intracellular sodium content may be kept low. There is increasing evidence that intracellular sodium determines levels of intracellular calcium by a control exerted at the mitochondria[14–16]. Increases in intracellular calcium may well be unacceptable in a tight epithelium if they lead to a loss of cell coupling[17].

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Endogenous C-type viral expression in primates

C-TYPE viruses are genetically transmitted in species representing several orders of vertebrates (for review see refs 1 and 2). So far the isolation of endogenous viruses of primates has been limited to species of *Papio*[3–5], although related viral nucleic acid sequences have been detected in cellular DNAs of several other Old World monkeys[6]. The extent of divergence of known endogenous primate viruses should be useful in predicting properties of the gene products of evolutionarily related endogenous viruses of primates from which such viruses have yet to be isolated. We report here an investigation of the antigenic divergence of different structural proteins of prototype endogenous primate C-type virus isolates. Endogenous viral antigen expression was also examined in tissues of primate species, representing the major genera of the order Anthropoidea. Immunologically divergent gene products of endogenous viruses were found to be expressed in tissues of Old World monkeys, including many species that have not yet yielded complete virus. These findings, together with the marked variations in viral antigen expression demonstrated among different primate genera, have implications for endogenous virus regulation in primates and the search for related viruses of humans.

The development of immunoassays for the 30,000 molecular weight major structural protein (p30)[7,8], the 70,000 molecular weight glycoprotein (gp70)[8] and a 15,000 molecular weight protein (p15)[9] of a prototype virus isolate of *P. cynocephalus* made it possible to compare the immunological relatedness of several endogenous primate viruses. Known C-type viral proteins contain a range of antigenic determinants that vary in their degree of cross-reactivity with analogous proteins of viruses of other species. In competition immunoassays using a given ^{125}I-labelled viral protein with limiting antiserum prepared against the same virus, the extent to which another virus can compete reflects the immunological relatedness of analogous proteins of the two viruses. As Fig. 1 shows, *P. cynocephalus* virus competed with high efficiency and to a final extent of more than 95% in immunoassays for three of its structural proteins. Endogenous viruses of *P. hamadryas* and *P. anubis* reacted as efficiently as *P. cynocephalus* virus in the immunoassay for *P. cynocephalus* virus in the immunoassay for *P. cynocephalus* virus p30 (Fig. 1*a*). In the assay for *P. cynocephalus* viral gp70, the other baboon viruses competed only as much as 80% (Fig. 1*b*), while neither competed more than 50% in the *P. cynocephalus* virus p15 immunoassay (Fig. 1*c*).

Certain endogenous viruses of the cat have been reported to be of primate origin and to have secondarily entered the cat lineage several million years ago[10,11]. Figure 1 demonstrates greater antigenic divergence of proteins of evolutionarily related feline viruses from those of the *P. cynocephalus* virus. Endogenous viruses of *Felis catus* and *F. sylvestris* showed significantly less ability to compete than the baboon viruses in the *P. cynocephalus* virus p30 assay (Fig. 1*a*) and even more striking antigenic divergence from *P. cynocephalus* virus in their p15 and gp70 reactivities (Fig. 1*b* and *c*). The relative conservation of p30 antigenic determinants suggests stronger evolutionary selective pressures for maintenance of the immunological integrity of this protein. Whether this also reflects preservation of some important biological function(s) is not resolved. In any event, it can be seen that the immunoassay for *P. cynocephalus* viral p30 was most broadly-reactive and thus most likely to be useful in a search for antigenic reactivity of related viruses in primate tissues.

Previous studies have demonstrated the expression of endogenous C-type viral antigens in normal tissues of vertebrates including the mouse[12–14], cat[15] and rhesus monkey[9,15]. In a systematic search for C-type viral gene products in tissues of a wide range of Old World monkeys, liver cell extracts were analysed for expression of antigens cross-reactive with *P. cynocephalus* viral p30. Cell extracts were subjected to DEAE–cellulose chromatography in an effort partially to purify p30 and reduce nonspecific reactivity[9].

As Table 1 shows, antigens cross reactive with *P. cynocephalus* viral p30 were detectable in tissues of species representing each of five genera of Old World monkeys (Table 1). The specificity of the antigenic reactivity was indicated by lack of competition in immunoassays for the p30s of other mammalian C-type viruses, including mouse and woolly monkey isolates (data not shown). Partially purified p30 antigens were subjected to molecular size analysis by gel filtration chromatography in both denaturing (6 M GuHCl) and non-denaturing conditions. In each case, tissue-associated p30 reactivity chromatographed with a molecular weight of about 30,000 relative to standards (data not shown). This sizing step generally provided a further 8–10-fold purification (Table 2) The degree of purification was sufficient to allow detection of p30 related to *P. cynocephalus* virus at a level about 50 times lower than that present in macaque tissues. The same procedures failed to reveal evidence of similar antigenic reactivity in more than 100 individual tissues analysed from higher apes or humans (Table 1).

As Table 1 shows, species representing two primate genera, *Papio* and *Macaca*, exhibited marked differences in their tissue expression of viral p30. Macaque tissues had relatively high p30 levels, from 15 to more than 100 ng per mg cellular protein. In most cases, baboon tissues had levels at least 20 times lower. Attempts to isolate endogenous C-type viruses from macaques have been unsuccessful. Yet, in similar conditions such viruses have been obtained readily from most species of baboon (refs 3–5 and our unpublished observations). This could reflect the release from macaque tissues of viruses with very different host ranges from those of known baboon endogenous viruses. Alternatively, the high levels of endogenous viral p30 expressed by macaque tissues in the absence of detectable virus release could be due to partial expression of a defective viral genome or to a late cellular block to release of a competent virus.

In view of the highly-conserved nature of p30 antigenic

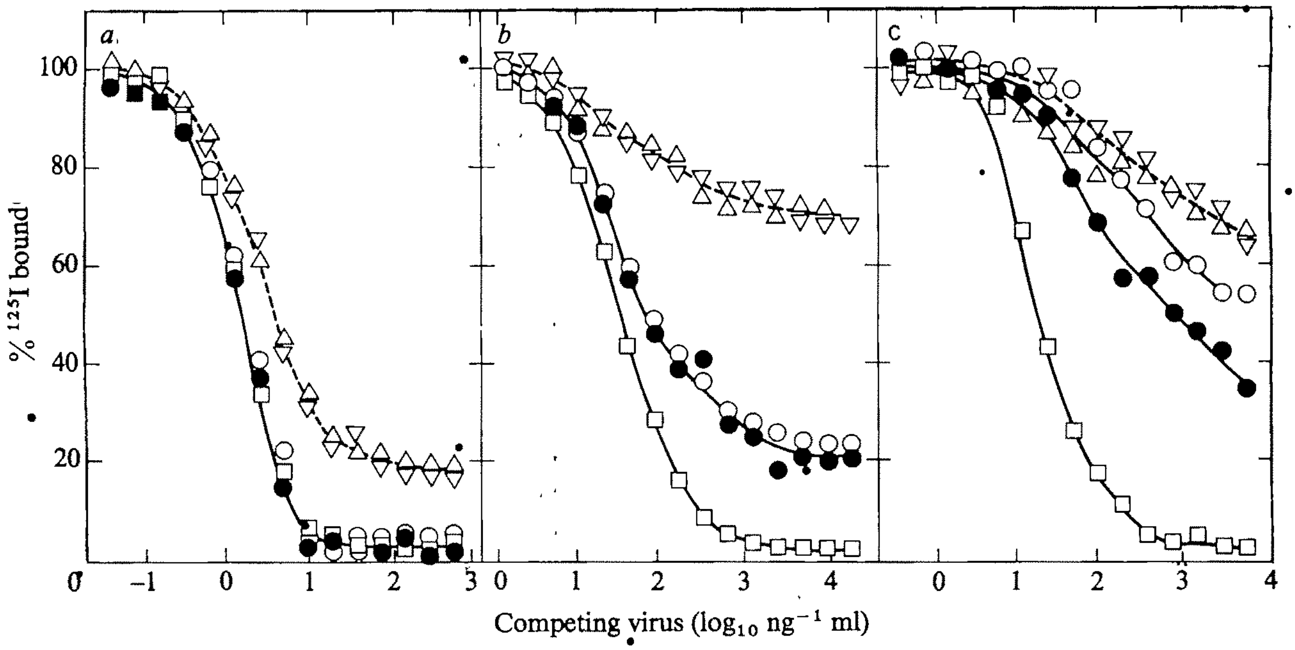

Fig. 1 Immunological divergence of viral structural proteins of endogenous primate C-type viruses Competition immunoassays were performed as described in Table 1 and included assays in which antiserum to the *P. cynocephalus* virus was used to precipitate ^{125}I-labelled *P. cynocephalus* viral p30 (*a*), gp70 (*b*) and p15 (*c*). Viruses, concentrated from tissue culture fluids by sucrose density centrifugation, were disrupted by incubation in 1% Triton X-100 at 37 °C for 30 min. Viruses tested included isolates of *P. cynocephalus* (□); *P. hamadryas* (○), and *P. anubis* (●); and endogenous viruses of two cat species, *F. catus* (△) and *F. sylvestris* (▽) The isolation of *P. cynocephalus*[3], *P. hamadryas*[5] and *F. catus* viruses[10] have been described. *P. anubis* and *F. sylvestris* viruses were isolated in our laboratory from normal kidney tissues of these species.

Table 1 Expression of antigen related to that of the major structural protein (p30) of *P. cynocephalus* virus

Family	Genus	Species	Common name	Viral p30 antigenic reactivity		
				No. tested	No. positive	Range (ng p30 per mg cellular protein)
Cercopithecidae	*Cercocebus*	*C. atys*	Sooty white-crowned mangabey	1	0	<1
	Cynopithecus	*C. niger*	Celebes (black) ape	1	1	10
	Papio	*P. cynocephalus*	Yellow baboon	1	1	2
		P. anubis	Olive baboon	1	1	3
		P hamadryas	Hamadryas baboon	1	0	<1
		P. papio	West African baboon	2	0	<1
		P. sphinx	Mandrill	1	0	<1
	Cercopithecus	*C. neglectus*	De Brazzas guenon	1	1	20
		C. aethiops	Grivet monkey	1	0	<1
		C. patas	Patas monkey	2	0	<1
	Macaca	*M. mulatta*	Rhesus macaque	10	10	15–110
		M. arctoides	Stump-tailed macaque	2	2	40–60
		M. nemestrina	Pig-tailed macaque	2	2	25–70
		M. irus	Crab-eating macaque	3	3	30–80
Pongidae	*Pongo*	*P. pygmaeus*	Orangutan	4	0	<1
	Pan	*P. troglodytes*	Chimpanzee	14	0	<1
	Gorilla	*G. gorilla*	Gorilla	1	0	<1
	Hylobates	*H. lar*	Gibbon ape	3	0	<1
Homoinidae	*Homo*	*H. sapiens*	Man	119	0	<1

Liver cell extracts were prepared by homogenisation in an equal volume of 0.01 M Tris-HCl, *p*H 7 8, 0 1 M NaCl, 0.5 mM EDTA and 0.5% Triton X-100, and sonication for 1 min with a Biosonic II sonic oscillator. Supernatants were clarified by centrifugation for 1 h at 100,000*g*, and subjected to partial purification by DEAE ion exchange chromatography[8]. Both original and DEAE-purified cell extracts were tested at serial two-fold dilutions for ability to compete with ^{125}I-labelled *P cynocephalus* virus p30 (ref. 10) for binding limiting concentrations of goat antisera to detergent-disrupted *P cynocephalus* virus. Antiserum and competing antigen were incubated at 37 °C for 1 h in 0.15 ml of reaction mixture containing 10 mM Tris-HCl, *p*H 7.8, 10 mM EDTA, 0.4% Triton X-100, 1% bovine serum albumin, 0.1% normal goat serum, 10 mM NaCl and the protease inhibitor phenylmethane-sulphonyl fluoride (300 μg ml^{-1}, Pierce Chemicals). About 10,000 c.p.m. ^{125}I-labelled p30 in 0 05 ml of the same buffer was added and incubation continued for 3 h at 37 °C and a further 18 h at 4 °C. Antigen–antibody complexes were precipitated by pig antigoat IgG[8] and radioactivity in the precipitates was measured in a Searle gamma counter Model 1285. Levels of p30 antigen in partially-purified liver cell extracts were calculated on the basis of the displacement of the competition curve for the unknown relative to that achieved with purified viral antigen. Protein concentrations were determined according to the method of Lowry *et al.*[23]. Results expressed on the basis of p30 concentrations in the original extracts were determined by quantifying p30 levels in DEAE-purified extract and correcting for the degree of purification achieved.

determinants among baboon endogenous viruses, it was interesting to investigate the extent of immunological relatedness of endogenous viral p30s among other genera of Old World monkeys As Fig. 2 shows, p30 antigens partially purified from liver tissues of *Macaca mulatta, Cynopithecus niger*, and *Cercopithecus neglectus*, each representing a different genus, competed less efficiently than that of p30 partially purified from *P. cynocephalus* liver tissue in the immunoassay for *P. cynocephalus* virus p30. These findings demonstrate that endogenous viral p30s of species representing different genera of Old World monkeys have undergone sufficient evolutionary change to make them immunologically distinguishable.

Table 2 Partial purification of antigen related to the *P cynocephalus* viral p30 from cell extracts of representative Old World monkey species

Genus	Viral p30 antigenic reactivity (ng per mg cell protein) after AcA54 Ultragel chromatography
Macaca	
M. mulatta	920
M arctoides	610
M. nemestrina	700
M. irus	950
Cynopithecus	
C. niger	280
Cercopithecus	
C. neglectus	150
Papio	
P. cynocephalus	40
P. anubis	28

P30 antigenic reactivity was purified partially from liver cell extracts by DEAE ion exchange chromatography as described in Table 1. Extracts were then subjected to molecular size analysis by AcA54 Ultragel column chromatography[24] and tested by competition immunoassay for *P cynocephalus* viral p30 reactivity as in Table 1. Results are expressed as ng p30 per mg cell protein after purification. Immunological specificity was established by absence of competition (<1 ng per mg cell protein) in immunoassays for mouse leukaemia virus and for a C-type virus of the woolly monkey[11].

The finding of gene products related to *P. cynocephalus* viral p30 in normal tissues of many species of Old World monkeys, together with previous molecular hybridisation studies[6], indicate the widespread distribution and expression of related endogenous viral sequences in these primates. In our studies, analogous translational products were not detected in tissues of higher apes or humans. Our findings that endogenous viral p30s of other Old World primate genera can be immunologically distinguished from those of baboons indicate that p30 of an evolutionarily related human endogenous virus would probably be distinguishable as well. Although other possibilities can be considered, previous claims of the detection of p30, indistinguishable from that of *P. cynocephalus* virus in certain human tumours[16,17], seem to favour infection of humans with baboon C-type virus. Such a model has been invoked[18,19] to explain the detection in certain human tumours of highly related nucleic acids[18,19] and even infectious virus[20], so far indistinguishable by the present techniques from *P. cynocephalus* virus[21]. The absence of viral antigens of the baboon virus in a large number of human tissues, as reported here, suggests that productive infection with a baboon C-type virus is likely to be infrequent if it occurs at all in humans.

The demonstrated presence of endogenous viral information in many species of Old World monkeys suggests that evolutionarily-related viruses exist in higher primates as well. Reports of low levels of genetic sequence homology between *P. cynocephalus* virus and normal cellular DNAs of apes and humans[6] would favour this possibility. The

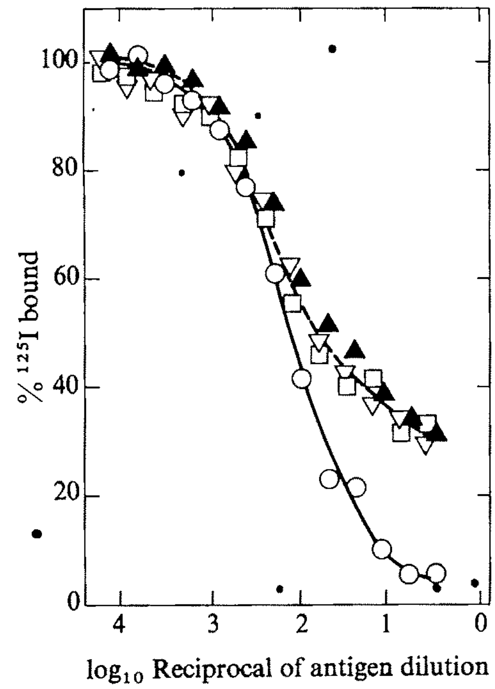

Fig. 2 Immunological divergence of endogenous viral p30 antigens partially purified from normal liver tissues of species representing different genera of the primate subfamily, Cercopithecinae. Liver cell extracts were subjected to DEAE-cellulose ion exchange and AcA54 Ultragel chromatography before analysis in a p30 competition immunoassay utilising antiserum to the *P. cynocephalus* virus precipitate, ^{125}I-labelled *P. cynocephalus* viral p30. Reactivity of partially-purified p30s from *P. cynocephalus* (○); *M. mulatta* (□); *C. niger* (▼); and *C. neglectus* (△).

absence of antigenic reactivity related to *P. cynocephalus* virus in these tissues implies that endogenous viruses of apes and man are either very tightly restricted or have undergone sufficient antigenic divergence to have become undetectable by available immunological techniques. Knowledge that immunoassays can recognise antigenic relatedness between endogenous viruses that have diverged to the extent that nucleic acid sequence homology is no longer demonstrable[21], justifies further efforts to develop more broadly-reactive and sensitive immunological probes to search for endogenous virus expression in man.

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Muscle histology and creatine kinase levels in the foetus in Duchenne muscular dystrophy

X-LINKED Duchenne muscular dystrophy is a serious genetic disorder for which at present there is no effective treatment. It is characterised by progressive muscle wasting and weakness which first becomes apparent around the age of 3–5 years, leading to death usually before the age of 20 (ref. 1) A proportion of female heterozygous carriers have significantly elevated serum levels of creatine kinase and a woman who is found to be at high risk of having an affected son can elect to have amniocentesis with antenatal foetal sexing and selective abortion of any male foetus. A proportion of such aborted foetuses will, however, be normal but since the cause of the disorder is not known it is not yet possible to diagnose an affected male foetus *in utero*. As a first step in this direction we have examined the muscle histology and levels of creatine kinase in serum, muscle and amniotic fluid in a number of therapeutically aborted male foetuses at risk for Duchenne muscular dystrophy. We report that muscle histology, and possibly the serum level of creatine kinase, may be abnormal in this disorder even by the second trimester of pregnancy.

Thirteen at-risk male foetuses were studied. In four cases (993, 1018, 75/479 and B105) the mothers were proven carriers and in the remaining cases the mothers were estimated to have a risk greater than one in five of being carriers (Table 1). In each case the sex of the foetus was established before abortion on the basis of cytogenetic studies on amniotic fluid cells obtained at amniocentesis As controls, 13 normal male foetuses of comparable gestation were obtained at abortion performed for social reasons where there was no family history of any neuromuscular disorder. The gestational ages of the foetuses were estimated by crown–rump and heel–toe measurements[2]

Cryostat sections of quadriceps muscle were stained with haematoxylin and eosin Muscle fibre diameters were measured using a calibrated Reichert Visospan projection microscope at a magnification of ×400. For each foetus 200 randomly chosen fibres were measured. The percentage of hyaline fibres was determined in 500 muscle fibres The cross-sectional areas of 100 randomly chosen nuclei, closely opposed to the surface of transversely sectioned muscle fibres, were estimated by planimetry at a final magnification of × 1000 Serum specimens were obtained by cardiac puncture and serum, cell-free amniotic fluid and muscle creatine kinase activities were measured according to the method of Rosalki[3] Creatine kinase activity in muscle was expressed in terms of total protein[4].

In affected boys muscle histology is characterised by variation in muscle fibre size, fibre necrosis and phagocytosis, and ultimately replacement by fat and connective tissue Muscle nuclear size is also increased[5]. Even in the preclinical stage of the disorder muscle histology is abnormal with hyalinisation of muscle fibres and increased variation in muscle fibre size[6–8]. In none of the at-risk foetuses was there any evidence of muscle fibre necrosis or phagocytosis or any increased amount of fat or connective tissue. Values outside the normal range, however, were found in six of the foetuses in regard to mean muscle fibre diameter and in five of the

Table 1 Mean muscle fibre diameter, variance in fibre size, percentage hyaline fibres, mean muscle nuclear size and serum levels of creatine kinase (SCK)

	P	Gestation (weeks)	Fibre diameter Mean (μm)	Fibre diameter Variance (σ^2)	Hyaline fibres content (%)	Mean nuclear size (μm^2)	SCK (IU)
Normal foetuses	—	14–21	6.9–10.7	0.8–3.6	0.0–0.6	22.1–34.1	120–7350
'At-risk' foetuses							
599	0.65	21	9.7	1.4	0.0	*37.1**	730
684	0.25	16	*12.4*	*6.8*	*2.5*	31.1*	*9140*
734	0.25	16	7.8	7.0	0.0	*40.8**	—
993	1.00	20	7.4	1.4	0.0	33.2	—
1018	1.00	21	7.2	0.5	0.0	34.1*	1220
73/375	0.21	19	5.8	2.0	0.0	*35.9**	6800
75/479	1.00	18	8.2	1.4	0.0	28.1*	1660
76/60	0.33	21	*13.5*	*9.0*	*2.0*	*39.8**	6100
B105	1.00	20	*12.4*	*12.3*	0.5	29.0	2700
878	0.23	17	*11.3*	*6.6*	0.2	27.3	5630
76/18	0.23	20	*10.9*	*5.6*	0.0	33.4	—
B107	0.25	14	*12.3*	2.9	0.5	30.4	—
B109	0.25	20	10.2	2.7	0.0	31.0	1500

In normal foetuses ($n = 13$) the results are expressed as the range; in at-risk foetuses individual results are given and italicised when outside the normal range. *P*, probability of a mother being heterozygous. *See ref. 12.

foetuses in regard to variance (σ^2) in fibre size (Table 1). There were very few hyaline fibres in normal foetuses (0.0–0.6%) but in two of the at-risk foetuses 2% or more of the muscle fibres were hyalinised, a finding which we have noted previously in a foetus at risk for Duchenne muscular dystrophy[9]. The mean muscle nuclear size seemed to be increased in four of the at-risk foetuses.

The serum level of creatine kinase is very high in the early stages of Duchenne muscular dystrophy and is moderately raised in infancy and even at birth[10,11]. In both normal and at risk foetuses there was considerable variation in serum levels of creatine kinase. The range in normal foetuses was 120–7350 IU and only one of the at risk foetuses (684) had a level outside this range (9140 IU). Creatine kinase levels in normal amniotic fluid were at the limit of accurate determination and no raised levels were found in any of the at-risk foetuses. Muscle creative kinase levels were within the normal range (1.3–9.6 IU per mg protein) in all of the at-risk foetuses.

Not all of the at-risk foetuses in this study would have been expected to be abnormal. In fact from pedigree data and maternal serum levels of creatine kinase the average probability of the mothers being heterozygous was 0.51 and therefore of the 13 at-risk foetuses it might be expected that (13) (0.5) (0.51) or 3.3 might be affected. In fact, using three or more criteria of abnormality, two foetuses could be classified as being affected, one of these (684) having a raised serum level of creatine kinase. Our results suggest that X-linked Duchenne muscular dystrophy is expressed, at least in muscle tissue, by the second trimester of pregnancy.

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Degradation of deadenylated rabbit α-globin mRNA in *Xenopus* oocytes is associated with its translation

THE 3′-OH poly(A) segment of globin mRNA ensures the stability of the message when injected into *Xenopus* oocytes[1–4]. We recently developed[5] a specific method for the removal of the poly(A) tail from globin mRNA, based on synchronous processive phosphorolysis of mRNA using molar excess of *E. coli* polynucleotide phosphorylase at 0 °C. When injected into *Xenopus* oocytes, deadenylated globin mRNA is translated for a relatively short period and then rapidly degraded[1,2]. Native poly(A)-containing mRNA, however, is considerably more stable in the same conditions and is translated for extended periods of time[1,2,7]. The poly (A) segment itself is responsible for the stability of native globin mRNA in *Xenopus* oocytes since poly(A) re-addition to previously deadenylated mRNA restores the functional stability of the message[3]. We do not yet know, however, how poly(A) exerts its protective function; furthermore, the mechanism of degradation of poly(A)-free mRNA is not known. We report here the use of the enhancing effects of haemin on the translation of α-globin mRNA in frog oocytes[6] to establish that, in these cells, the degradation of injected poly(A)-free α-globin mRNA is linked to its translation.

Rabbit α-globin mRNA is much less efficiently translated than β-globin message when injected into frog oocytes. The efficiency of α-globin mRNA translation can, however, be dramatically increased if haemin is included in the injection mixture[6]. The stimulation of α-globin mRNA translation by haemin is shown in Fig. 1. We took advantage of this effect to determine whether the degradation of deadenylated α-globin mRNA is linked to its translation.

We examined the effect of haemin on the degradation of poly(A)-free rabbit α-globin mRNA injected into *Xenopus* oocytes. Rabbit globin mRNA enriched in α message was prepared from the post-ribosomal supernatant of reticulocytes lysate[8,9]; some was used as a template for the synthesis of a αH^3-cDNA probe by a reverse transcriptase reaction[10] and the remainder was deadenylated by processive phosphorolysis with polynucleotide phosphorylase[5]. Two samples of 100 oocytes from the same *X. laevis* female were microinjected[11] with an aqueous solution of poly(A)-free α-globin mRNA with or without haemin. Forty oocytes from each batch were stored immediately after injection and the remaining 60 oocytes of each batch were incubated for 72 h in

Barth medium at 20 °C. We then used molecular hybridisation with the ^{3}H-cDNA probe to compare the amount of globin mRNA remaining in oocytes microinjected with either poly(A)-free mRNA alone and with haemin. Increasing amounts of RNA from each sample of oocytes were hybridised to a constant amount of α-globin enriched ^{3}H-cDNA at concentration where less than 20% of the radioactive probe is annealed at the end of the reaction[2]. In these conditions, the amount of ^{3}H-cDNA annealed was proportional to the amount of globin mRNA present in the reaction medium for all samples tested (Fig. 2). The slopes of the straight lines corresponding to the four samples of oocytes thus constitute relative measurements of the amounts of globin mRNA present in each batch.

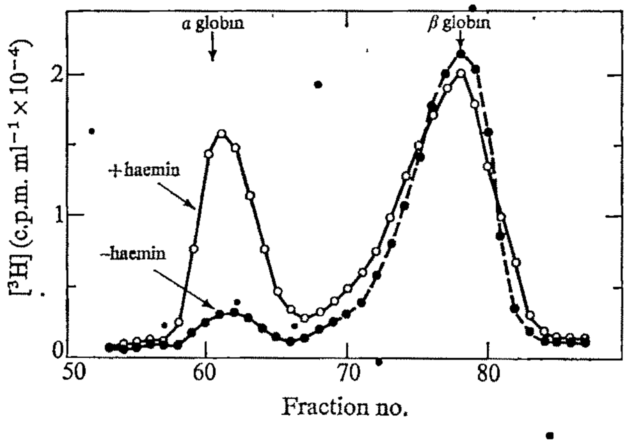
In the presence of haemin only 13% of the injected poly (A)-free α-globin mRNA remained intact at the end of the incubation, whereas 60% of the injected message remained hybridisable to the ^{3}H-cDNA probe in the absence of haemin (Fig. 2). Similar results were obtained in another experiment using different preparations of α-globin mRNA and H^3-cDNA as well as oocytes from a different frog.

From previous experiments[3] and controls data (not presented), it is known that haemin *per se* has no degradative effect on injected native globin mRNA. Thus, the simplest interpretation of our results is that, in *Xenopus* oocytes there is a mechanism for the degradation of poly(A)-free mRNA requiring or strongly activated by translation or at least its initiation.

Puromycin enhances the degradation of a microinjected *Dictiostyleum* RNA preparation enriched in mRNA[12]; this led to the suggestion that the release of mRNA from ribosomes reduces its stability. We cannot explain these results as, using purified native globin mRNA in the same kind of experiments, we did not observe any effect of puromycin on the stability of the message. It should be noted, however, that it has proven impossible to keep oocytes in healthy conditions for long periods of puromycin treatment. One may therefore question the physiological meaning of such experiments. The use of haemin to enhance the translation of α-globin mRNA does not have this disadvantage.

On the other hand, it has recently been claimed[13,14] that translation, or at least some of the step(s) involved in the process, are required for the degradation of mRNA in different systems. The observations presented here strengthen these claims.

Fig. 1 Relative synthesis of α-and β-rabbit globin chains in oocytes injected with native globin mRNA 5 ng per 50 nl. ●, In the absence of haemin; ○, in the presence of haemin (1.5 mM in the injection medium). Following injection, each set of oocytes was incubated for 15 h at 20 °C in Barth's medium containing ^{3}H-histidine (50 Ci mol^{-1}, 200 μCi ml^{-1}). After incubation, the oocytes were homogenised with carrier rabbit haemoglobin and globin was prepared from the 10,000*g* supernatant of the homogenate[15]. Globin chains were separated on carboxymethyl-cellulose columns by a pyridine–formic acid gradient[15] and the radioactivity of the eluted fractions was determined in a scintillation spectrometer.



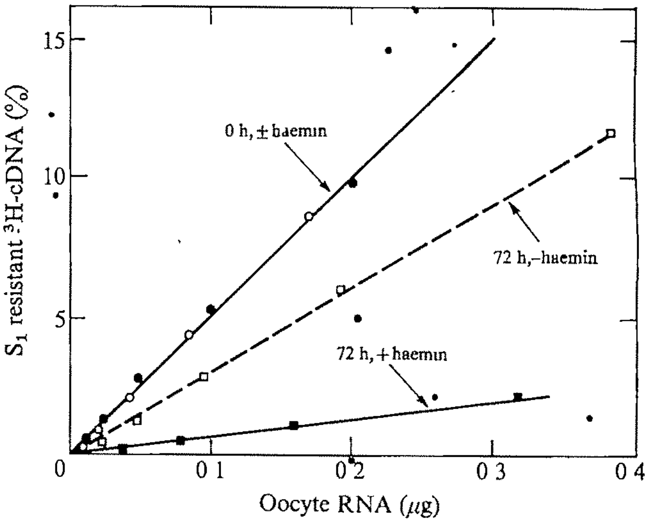


Fig. 2 Effect of haemin on the stability of poly(A)-free α-globin mRNA in *X, laevis* oocytes. Two hundred *Xenopus* oocytes were injected with poly (A)-free α-enriched globin mRNA 5 ng per 50 nl in aqueous solution; 100 oocytes were injected with mRNA in the absence of haemin, and a further 100 were injected with mRNA dissolved in a 1.5 mM haemin solution. Forty oocytes from each batch were frozen immediately after microinjection: the remaining 60 were incubated in Barth's medium at 20 °C for 72 h. Total RNA from each sample of oocytes was extracted using a phenol–chloroform procedure[4]. Increasing amounts of RNA from each sample of oocytes were hybridised to a fixed amount of globin ^{3}H-cDNA in conditions where the amount of annealed cDNA is proportional to the amount of globin mRNA present in the reaction medium. Hybrids were detected by their S$_1$ nuclease resistance[5]. Hybridisation of RNA to (^{3}H) globin cDNA: □, ○, RNA was extracted from oocytes micro-injected with poly (A)-free α-enriched globin mRNA without haemin; ○, RNA extracted immediately after injection; □, RNA extracted after 72 h of incubation of oocytes. ■, ●, the same with haemin.

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matters arising

Backwarming due to circumstellar shells

IN a recent publication, Donnison and Williams[1] discussed the possible effects which a circumstellar dust shell could have on the evolution of a premain sequence star surrounded by it. If λ is the fraction of energy emitted by the star that returns back to it, then they concluded that $(1-\lambda)/\lambda$ can have a value as low as

$$(1-\lambda)/\lambda \simeq (T_O/T_I)^4 \quad (1)$$

where T_I and T_O represent the temperatures of the inner and the outer surfaces of the shell. We intend here to draw attention to the fact that equation (1) could be misleading since it represents the smallest value that can ever be allowed by the ratio, and is by no means a realistic limit. The full expression for this ratio can simply be derived as follows.

If we assume that the shell stores no energy, then

$$R^2T^4_{\mathrm{eff}}(1-\lambda) = \alpha_1 R^2_O T^4_O \quad (2)$$

where R and R_O are the stellar radius and outer radius of the shell and T_{eff} is the effective temperature of the star, α_1 is a constant, which is included because the outer surface in reality will not be a perfect black body characterised by the temperature T_O.

Simple geometry implies that the fraction of the radiation emitted by the inner surface which strikes the star is

$$\alpha_2 \mu R_I^2 T_I^4 \quad (3)$$

where α_2 compensates for the inner surface not being a perfect black body and μ is the fraction of the total solid angle which is subtended by the star at the inner surface of the shell, so that $\mu = \beta(R^2/R_I^2)$ with β a numerical constant which is easy to determine if the star is small but more complicated if the star and shell have similar radii. Hence, from (3)

$$\lambda R^2 T^4_{\mathrm{eff}} = \alpha_2 \beta R^2 T_I^4 \quad (4)$$

From (2) and (4)

$$\frac{(1-\lambda)}{\lambda} = \left(\frac{\alpha_1 R_O^2}{\alpha_2 \beta R^2}\right)\left(\frac{T_O}{T_I}\right)^4 \quad (5)$$

And it is immediately evident that equation (1) as used in the reference[1] is only applicable to a very restricted set of physical parameters where

$$\alpha_1 R_O^2 = \alpha_2 \beta R^2 \quad (6)$$

In general $R_O \gg R$ and the ratio $(1-\lambda)/\lambda$ is very much greater than the value given by equation (1) (that is, λ is smaller).

We conclude, therefore, that even though it is theoretically possible to have a situation where λ is close to unity, which results in a drastic change in the evolution of the central star, generally, in physical meaningful situations $\lambda \ll 1$ and backwarming has no real effect.

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Possible solar control of North Atlantic oceanic climate

TRENDS in the climate of the North Atlantic Ocean over the past century have been described recently by Colebrook[1]. He discussed the variation in the average sea level, the number of cyclones and the sea-surface temperature. He showed that low sea-surface temperatures in the North Atlantic are associated with a higher-than-normal flow in the Gulf Stream and a lower-than-normal frequency of tropical cyclones, indicative of above average intensity in mid-latitude westerlies. The sea-surface temperature data were smoothed by the application of eigenvector filters which were approximately equivalent to 7-yr running means. A 10–11-yr periodicity, which tended to be in phase with the sunspot cycle, was found following the removal of long-term trends from the smoothed data. I point out here a very significant long-term correlation between the sea-surface temperature and the 7-yr running mean of the annual sunspot numbers. I have redrawn the sea-surface temperature data of Colebrook[1] and compared it with the 7-yr running mean of the annual sunspot number for Marsden squares 145 D and 182 B (Fig. 1). In both cases the vertical scales were adjusted so as to make the total amplitude of the long-term variation approximately the same on the graph.

There is very good correlation between the two sets of data for Marsden square 145 D (45°–50°N, 5°–10°W) while the correlation is less marked for Marsden square 182 B (50°–55°N, 15°–20°W). This difference in the degree of correlation between the two sets of data might be accounted for by the difference in latitude between the two areas under consideration. The effect of latitude on the

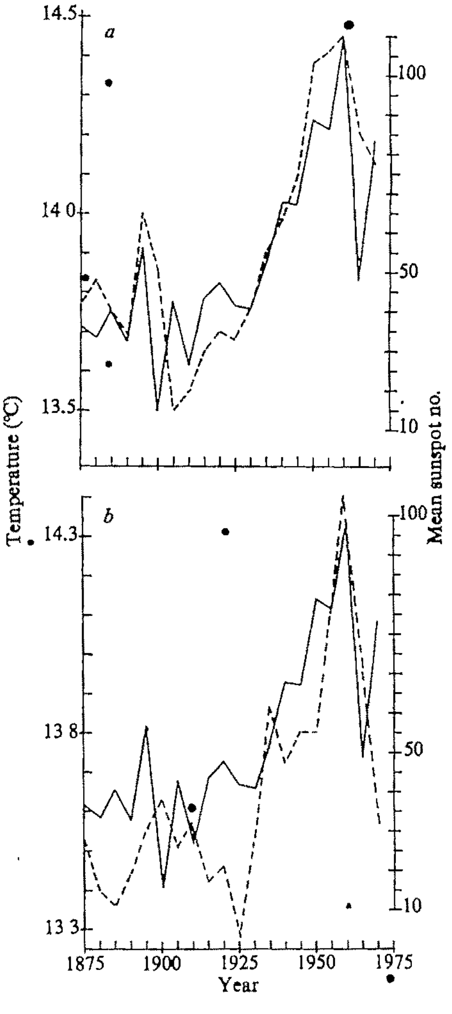

Fig. 1 Smoothed variations of sea-surface temperature redrawn from ref. 1 (broken lines) compared with the 7-yr running mean of the annual sunspot number (solid line). *a* Refers to Marsden square 145 D (45–50°N, 5–10°W) and *b* refers to Marsden square 182 B (50°–55°N, 15°–20°W).

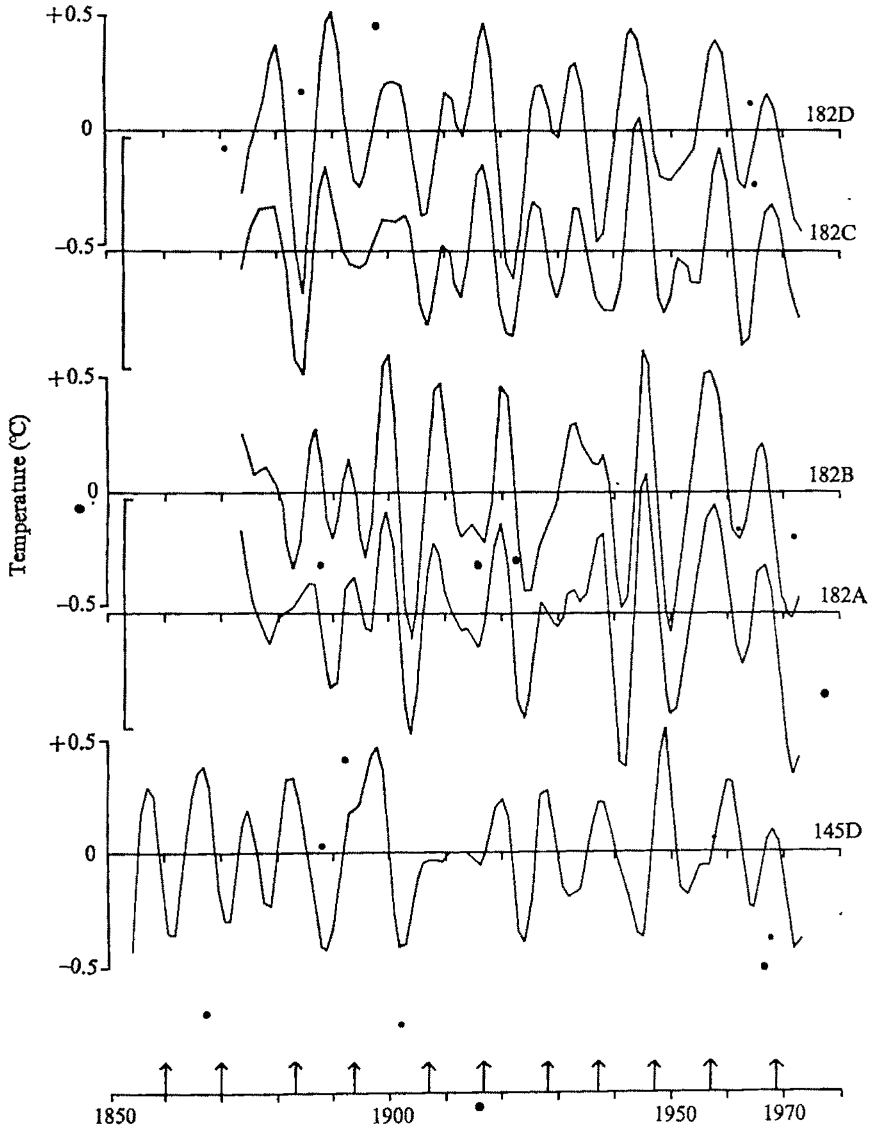

Fig. 1 Filtered mean sea-surface temperatures for quarter Marsden squares 145 (Celtic Sea and Bay of Biscay) and 182 (Rockall area). The variables show the dominant periodic element following the removal of long term trends. Arrows on abscissa indicate years of maximum sunspot numbers.

possible relationship between solar activity and the circulation of the lower atmosphere has been discussed at some length by King[2].

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Colebrook replies—In the light of the very impressive correlation between sea-surface temperature and annual sunspot numbers demonstrated by Muir[1], I comment here in greater detail than was possible before[2] on the periodicities in sea-surface temperature data for Marsden squares 182 and 145. Figure 1 shows plots of filtered sea-surface temperatures representing the dominant periodic elements in the series following the removal of the long term trend. In each case there is a marked 10–11-yr periodicity. There is a fairly good phase relationship between the various temperature series and with the sunspot maxima

It should also be noted that Cohen and Sweetser[3] detected an 11-yr periodicity in their series of frequencies of tropical cyclones in the Atlantic.

In the light of all the evidence now available there seems little doubt that sunspot numbers do have an influence on the climate of the North Atlantic Ocean.

I suggested previously[2] that the effect on sea-surface temperatures in the north-east Atlantic was produced by variations in southerly anomaly winds, involving direct heat exchange with the atmosphere. The relationship found by Muir implies an influence on the atmospheric circulation over the whole North Atlantic with a consequent effect on the Gulf Stream. This is consistent with the results presented by Maximov[4] which indicated that the 10–11-yr cycle in sea-surface temperature occurs over most of the North Atlantic Ocean.

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Oxygen isotope ratios in spruce cellulose

Although not affecting the practical significance of their work, Gray and Thompson's[1] finding that oxygen isotope fractionation in the cellulose of white spruce (*Picea glauca*) depends on winter rather than summer temperature warrants further discussion. It is unlikely that the tree is photosynthesising to any significant degree in the winter. I suggest here an alternative mechanism which would produce the observed results.

Two facts argue against winter photosynthesis: First, Fritts's[2] conifer, cited by Gray and Thompson, photosynthesised only when the temperature was above freezing and soil water stress was low—two conditions unlikely to hold for long periods in the Edmonton winter. Second, Norway spruce (*Picea abies*) chloroplasts function in carbohydrate synthesis only during the summer months[3]. If this is also true for white spruce, winter photosynthesis is probably not possible.

For energetic reasons a heavier isotope accumulates preferentially in that molecule having the highest bond multiplicity at the point of isotopic substitution[4,5]. Thus, when ^{18}O and ^{16}O distribute themselves between H_2O and CO_2, the CO_2 (in which oxygen is double bonded) will get proportionately more ^{18}O than the H_2O (in which oxygen is single bonded). This effect decreases with increasing temperature[6]. Therefore, if one looks at oxygen from CO_2 which has been in solution with H_2O, the δ ^{18}O-temperature curve will have a negative slope. But, Gray and Thompson's curve has a positive slope. The oxygen in their cellulose might have come from H_2O rather than CO_2, but this is not consistent with our current understanding of photosynthesis. Alternatively, starch may be hydrolysed to glucose within the cell during the winter. The glucose (single-bonded oxygen) would then exchange oxygen with cellular organic acids (or any other molecules containing double or triple oxygen bonds), producing the observed δ^{18}O–temperature curve.

The behaviour of Norway spruce chloroplasts[3] seems to support this idea. In autumn, they function primarily in starch storage. In winter, they group together and membranes become disorganised. In spring, they again store starch—of necessity the same starch which was stored in the autumn. This mechanism suggests that the bulk of radial growth in the tree is produced by the previous year's photosynthate, which is contrary to accepted thinking[7].

Hydrolysis of starch to sugars in the winter could have functional value. Sugar solutions protect at least some cell functions during freezing[8]. Heat of hydrolysis could conceivably provide a significant warming effect within the cell.

I thank Dr James Pickett for helpful discussions.

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Isotope tree thermometers

WE wish to make several comments on the recent paper in *Nature* by Libby *et al.*[1]

They determine the δ^{18}O in wood by analysing the CO_2 produced by heating the total wood samples with $HgCl_2$.

$$\delta D‰ = \left(\frac{\text{D/H sample}}{\text{D/H standard}} - 1\right) 1000$$

The standard is usually standard mean ocean water (SMOW). δ^{18}O is defined similarly. The standard used in Libby *et al.*[1], is as given in their text. Neither Libby *et al.*[1] nor the original developers of this method[2] have shown that this procedure is valid for the high accuracy necessary in this work.

The δD data shown in their Fig. 3 (ref. 1) was originally published elsewhere[3] where they reported that these δD analyses were made on H_2 released by pyrolysis of a mixture of wood sawdust and powdered uranium. In their *Nature* article[1] they state that this same δD data were obtained by combustion of the wood with oxygen and passage of the resulting water over hot uranium. This apparent contradiction should be cleared up, since only the latter method has been shown to be reliable[4–6].

They assume that the δ^{18}O and δD values of the total wood can measure by proxy the δ^{18}O and δD of meteoric water used by the plants. It is the isotopic composition of the meteoric water which is the 'thermometer'. Their assumption is incorrect[6]. It is known[5,6] that the δD of lipids in the wood can be 100‰ lower than the δD of the total plant. Thus even if correct δD analyses are made on total wood, this δD also depends on the wood's variable chemical composition which is not temperature sensitive[6].

Their sole criteria for a valid relationship between temperature and the δD and δ^{18}O values of total wood is the existence of doubtful correlations between the isotope records in dated tree rings and the time-equivalent instrumentally recorded temperatures in cities. Their choice of climatic data for correlation purposes is subjective. For example, the isotopic data of their Fig. 1 is correlated with average annual temperature, whereas, in their Fig. 3 the correlation is between isotopic data and English winter temperature. Their data bear little correlation with the climatic history of Europe as estimated by Lamb[7].

Some of their data are very unusual. The δD for the German oak tree rings shown in their Fig. 3 changes by 150‰ between 1820 and 1940 θ⁻ and 190‰ between 1720 and 1820 θ⁻. This change is about three times as large as the δD change between summer and winter precipitation in Vienna in their Fig. 4, which corresponds to a 20 °C change in temperature. Also there is only a 180‰ range in the δD of average precipitation available to plants growing from tropical to subarctic temperatures[8] (Fig. 1 below). These changes in δD values in the oak tree could not be due to changes in δD of the precipitation in Germany nor to climatic changes. Similar questions can be raised about the unusually large changes in δ^{18}O in their Fig. 1[1].

We have made some δ^{18}O measurements on cellulose from tree wood and other plants (S. G., P. Thompson and C. J. Y., unpublished) and found that the relationship between the δD and δ^{18}O in cellulose is complicated by factors such as evaporation–transpiration in the plants. There is no *a priori* reason to expect the δD against δ^{18}O relationship to be similar to those for meteoric waters. Thus their claimed slope of 8 for the relationship between the δ^{18}O and δD for total wood shown in their Fig. 6, and the conclusions they draw from this figure are puzzling. The slope 8 is not by itself an indication that the tree is storing old water, nor does it support their stated hypothesis that atmospheric CO_2 and atmospheric H_2O are in isotopic equilibrium. The δ^{18}O of the atmospheric CO_2 varies little and is basically determined by the equilibration of CO_2 with ocean water[8–11].

Fourier analyses of a continuous 2,000-yr record of δD and δ^{18}O analyses for five-year intervals of tree rings should reveal the presence of shorter cycles than reported by them. It is difficult to understand why the shorter cycles were not included in their Fig. 7 considering their expressed interest in finding a 21-year cycle.

We conclude that the δD and δ^{18}O as determined by Libby, *et al.* on total wood do not give information about climate. On the other hand, we have shown that the δD analysis of the hydrogen from only the C–H groups in cellulose (isolated as nitrated cellulose) eliminates the problems of chemical heterogeneity and the exchange with water of the hydrogen in the OH groups[6]. The δD values of the cellulose C–H hydrogen and of the associated OH groups[6]. The δD values of the cellulos C–H hydrogen and of the ossociated environmental water were compared for 20 different species of land and aquatic plants including nine different species of trees, collected from areas including Scotland, the Yukon Territory, Wisconsin, California, Texas, and Miami, Florida. The comparison is shown in Fig. 1. The δD of the C–H hydrogen is, on the average −20±11% (1σ) relative to the δD of the environmental water. Thus, the δD of cellulose C–H hydrogen from plants systematically reflects the δD of the H_2O incorporated by them and should be a good climatic thermometer.

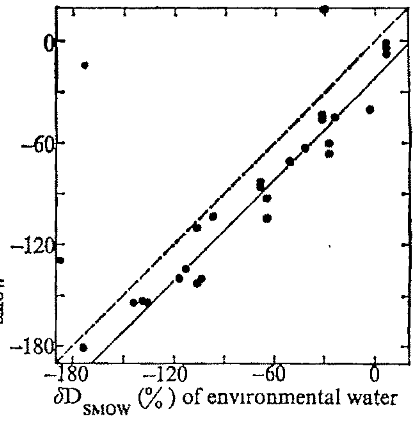

Fig. 1 The plot of δD of plant-derived cellulose C–H hydrogen (cellulose nitrate) against δD of environmental waters used in the plants, yields a linear regression curve (solid line) with a slope of 0 97 and intercept of −22‰. The linear correlation coefficient is 0.978. The dashed line is the $y = x$ curve. The samples include varieties of both land and aquatic plants from a wide geographic range.

Historically, Libby *et al.* should note that, by 1970, at least two laboratories[4,5] were measuring the isotopic composition of plants in order to determine by proxy the isotopic composition of meteoric water.

As far as the item on future research is concerned, we have already done much of what Libby *et al.*[1] recommend, including the identification of a 22-yr cycle in a 1,000-yr δD record[12] in the bristlecone pine of the White Mountains, California. We also analysed forty trees ranging in age from 9,000–22,000 yr to obtain a most interesting record of δD of meteoric water during the Wisconsin glacial period[13].

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LEONA MARSHALL LIBBY AND LOUIS J. PANDOLFI REPLY—Long-term isotope changes in precipitation, caused by changes in climatic temperature, are well documented in polar ice caps; the heavier of the stable isotopes is depleted in ice laid down in the ice age by comparison with present-day ice. In 1970 we extended this concept to trees, suggesting that they are also thermometers. In trees which use rain to manufacture their rings, isotope variations in the rings should be climate indicators because the isotope composition in rain and CO_2 varies with temperature.

On May 17 1971, the Advanced Research Projects Agency funded our proposal that "temperature variations may be evaluated by measuring stable isotope ratios in natural data banks such as tree rings and varves." L.M.L. had previously calculated[1] the theoretical temperature coefficient of the isotope fractionation in manufacture of wood from CO_2 and H_2O, finding that the coefficient is small compared with that measured in rain.[2] Therefore isotope variations in rain should be dominant in trees that drink rain.

The next question is what to measure: whole wood, cellulose, lignin . . . ? It may be true that the ratio of cellulose to lignin differs in spring and summer wood and over spans of decades.[3] Tentatively it seems that there is more lignin when it is warmer as in later summer, and that lignin is more enriched in the heavy isotope. We choose to regard such variations as part of the response of trees to climate variations, namely as part of the record of climate history.

Thus it is valid to measure any one of these substances, depending how faithfully it tracks local mercury thermometers. Our measurements on whole wood track for both hydrogen and oxygen. We expect the same success for cellulose and lignin, but they will track with different amplitudes because in these compounds different sets of chemical bonds are fractionating when the temperature changes.

In the development of radiocarbon dating the same question arose whether to measure wood, cellulose, or lignin, that is the $^{13}C/^{12}C$ and $^{14}C/^{12}C$ ratios will be different in each of these materials just as D/H is. W. F. L. chose to measure whole wood.

The next problem is what chemistry to use. There is compelling evidence that when sapwood passes into heartwood it becomes sealed against exchange with sap.[4] It is also known that isotope exchange occurs when wood is finely ground and soaked in hydrogen containing liquids[5]. So we decided to use dry chemistries. For our first tree sequence[6,7] we measured D/H by reacting sawdust with uranium to release H^2, 99‰ quantitatively. For the later tree sequences[4] we burned wood to water and reacted it with uranium to release H_2. For measurement of $^{18}O^{16}O$ we modified the method of Rittenberg and Pontecorvo[8] by carrying it out at very high temperatures, 99% quantitatively.

Our third problem was which trees to study. We could not use bristlecones because there are no lengthy temperature records for hundreds of miles near where they grow and we were determined to calibrate the trees against local mercury thermometers. Because the longest temperature records are in Europe, we obtained a German oak from the only tree laboratory then existing in Europe[4].

For measurement of the oaks, we used per force a mass spectrometer of somewhat low accuracy, and achieved the accuracy necessary to demonstrate tree thermometers by making many measurements on each sample. On the later tree sequences[4] we used high-precision machines with accuracies of ±0.1 p.p.t. for $^{18}O/^{16}O$ and ±2 p.p.t. for D/H. Whether the oxygen in tree rings comes from water or from CO_2 is a non-question, because Cohn and Urey[9] showed that isotopic equilibrium between the two substances is obtained in the atmosphere in a few hours.

The least square correlation of D/H against $^{18}O/^{16}O$ in the 1,800 yr sequence yielded the relation,

$$\delta D = 8.2\,\delta^{18}O + \text{constant}$$

which is the same as in worldwide rain[10] within experimental error. If both the hydrogen and the oxygen in the OH groups exchanged with water vapour there is absolutely no way that the slope can be 8, because there are twice as many hydrogens bonded to carbon as to oxygen.

The Fourier transforms[4] of our five-year samples in the 1,800 yr sequence showed ten significant periods ranging between 50 and 300 yr, the same for D/H and for $^{18}O/^{16}O$. To look for periods of less than 50 yr in a sequence of five-year samples or to look for periods of more than 300 years in a sequence of 1,800 yr is meaningless. Such periods appear in the transforms but they do not necessarily represent reality. To understand this we generated random signals of values 0 and 1, and assigned them to 10-yr intervals of a 1,000-yr hypothetical sequence, and made a Fourier transform. In three such computer experiments we always obtained periods at 10 and about 20yr which are artefacts of the sampling interval. These statements can be understood by reading carefully ref. 11, particularly pages 30–33.

Obviously ring samples integrated over 5 or 10 yr cannot give proof of the 21-yr solar cycle; instead each ring must be measured.

Nine of our ten periods[4] are equal to[12] those evidenced in the Greenland ice wells.[13] One of the periods, that at 173 yr, has also been found by Houtermans and Suess[14] in bristlecone pines.

Our isotope ratios track better with winter temperatures than with the average temperatures. Dr John Fletcher suggested that when climate deteriorates summer is brief and January, February, and March represent the longer winters.

For the cellulose component of wood, Epstein and Yapp[15] and Gray and Thompson[16] have corroborated our work.

Tree-ring thermometry promises to evaluate past climates and predict future climates.

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Chemotherapy of Herpes Simplex Virus

edited by J. S. Oxford, F. A. Drasar and J. D. Williams

May/June 1977, viii + 154pp., £4.80 (paperbound) 0.12.531760.3

Herpes virus infections of man are an important cause of ocular disease and virus encephalitis. In certain countries genital herpes is a sexually transmitted disease second only, in frequency, to gonorrhea. Virologists, clinicians, chemists, biochemists and molecular biologists have contributed to the text, as it is felt that progress in the control of the human virus depends on a multidisciplinary approach General aspects of herpes infection in man, domestic animals and birds are discussed, along with the basic virology. The usefulness of various laboratory model infections, in the search for new drugs, are examined and the few compounds used in clinical medicine are assessed.

Advances in Physical Organic Chemistry Volume 14

edited by V. Gold

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April/May 1977, viii + 376pp., £16.00/$31.25 0.12.033514.X

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Transactions of the Faraday Society

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Linnean Society Symposium Series Number 4

Problems in Vertebrate Evolution

edited by S. Mahala Andrews, R. S. Miles and A. D. Walker

April/May 1977, xii + 412pp., 25 plates £18.50/$36.10 0.12.059950.3

This book provides a new look at a wider range of problems in vertebrate evolution dealing, amongst others, with agathans, fishes, birds, dinosaurs and lizards, and including studies on both fossil and Recent species The contributors present new evidence and also novel interpretations of existing data. Their work covers specialised problems in vertebrate history involving comparative and functional anatomy, microstructure and morphogenesis The papers reflect the rapid changes that have taken place in comparative biology in the last decade as a result of new discoveries, the introduction of new techniques and refinement of methodology, and they indicate the possible direction of future work

The Chemistry of Pyrroles

R. Alan Jones and G. P. Bean

May/June 1977, x + 528pp., £21.00/$41.00 0.12.389840.4

The aim of this textbook is to provide a comprehensive survey of the basic chemistry of pyrrole presented in a systematic modern manner. The eleven chapters discuss the structure, synthesis, and the reactivity of the pyrrole ring, the synthesis and reactivity of substituted pyrroles and the physical organic properties of pyrrole and its derivatives This new and very comprehensive book will be of great value to undergraduate and graduate students and workers in the fields of chemistry and biochemistry, as well as in pharmacy and medicinal chemistry.

Rare Gas Solids Volume 2

edited by M. L. Klein and J. A. Venables

May/June 1977, xiv + 644pp., £26.00/$50.75 0.12.413502.1

This book, together with Volume I, gives an up-to-date account of research on the rare gas solids. These solids, formed by condensing helium, neon, argon, krypton and xenon, are the simplest of all molecular solids, and consequently both theoretical and experimental research has progressed further than that on other molecular solids By describing achievements, and highlighting points of uncertainty, it is intended to stimulate further research on all these materials. The present volume contains largely the experimentalists's view of the subject, in contrast to Volume I which was mostly written by theoreticians. Volume 2 is to some extent technique orientated and contains extensive tabulations and critical evaluation of experimental data for the rare gas solids

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reviews

Interferon as an anti-viral

Thomas C. Merigan

Interferon and Its Clinical Potential. By D. A. J. Tyrrell. Pp. viii+104. (Heinemann Medical: London, 1976.) £3.50.

THIS brief and timely monograph clearly presents the author's personal views of the interferon area. The 91-page review covers two decades of intensive research which has formed the basis for the present clinical investigations involving interferon, following its discovery in 1957. This remarkable molecule is not only of clinical interest as an anti-viral, but there is an emerging body of information supporting its role as a locally active cell regulator with a broad range of functions beyond its anti-viral action. It not only seems to influence malignant, immune and haematopoietic cell division, but certain specialised cell biosynthetic functions as well. This viral inhibitor discovered by Alick Isaacs now also attracts the efforts of molecular biologists as cell-free systems have been developed in which it can be produced and in which anti-viral action can be demonstrated. The recent discovery of cell membrane receptors for interferon, which seem to be gangliosides, should now allow elucidation of the mechanism of the first step in its action—that is, its binding to cells.

The book provides the clinician, basic virologist, and specialised investigator with a firm basis for understanding the potential of interferon in human medicine. It is digestable in an evening of solid reading by anyone with an understanding of modern biology. Although clinical trial results are appearing with greater frequency and views on its mechanism of action will be sharpened as more is learned of the fundamentals of viral replication, the basic description of the interferon system presented in this volume will be useful to future investigators.

The enthusiasm of the writer and his clear style of writing leads the reader through the voluminous recent literature in this area. One hundred and sixty-five carefully selected references are used to support a very readable discussion of interferon action and production in cell culture, animal models and human disease settings. Present concepts of the assay, properties and purification of interferon are also discussed. A number of well cast tables and easy-to-comprehend diagrams aid the reader in following the text. The methods for preparation and purification of human leukocyte interferon for clinical use, developed in Finland and in the UK, are discussed by the author, who was chairman of the UK Scientific Committee on Interferon, a group which planned and supported much of the early work with the material. The long experience of the author in this area insures the reader that the book accurately presents the basic biology of the interferon system, together with present accomplishments and perspectives for its use as a clinical anti-viral and anti-tumour agent. ☐

T. C. Merigan is Professor of Medicine and Head of the Division of Infectious Diseases at Stanford University School of Medicine, Stanford, California.

Arroyos in the American South-West

Arroyos and Environmental Change in the American South-West. By R. U. Cooke and R. W. Reeves. Pp. xii+213. (Clarendon: Oxford, 1976.) £8.50.

LARGE gullies with near vertical walls and flat, sandy bottoms, dissecting the alluvial valley floors of the American South-West, have attracted the attention of geologists and geographers for several decades. The arroyos, as they are called, seem to have developed after 1850, about the time of white settlement and the introduction of livestock in large numbers to the western plains. Not unnaturally, it was commonly supposed that land-use changes were responsible for the erosion, on account of depletion of the vegetation and greater runoff. Sedimentary sections showed, however, that there had been earlier periods of trenching in prehistoric times, and it was suggested that slight climatic changes were at least partly responsible.

It is known that overstocking occurred in Arizona about the same time as severe drought in the 1880s, and it is clear from J. R. Hastings and R. M. Turner in *The Changing Mile* (University of Arizona, Tucson, 1965) and from other sources that the vegetation has deteriorated markedly since that time. Cooke and Reeves show that in the coastal regions of southern California where arroyos also occur, rainfall records provide no support for the idea that secular or cyclic changes in precipitation have played a determining role in the formation of the arroyos. Furthermore, they present evidence that calls into question much that is commonly accepted about the effects of land-use on runoff coefficients.

Using archival records of various kinds, and in particular the field notebooks of the US General Land Office surveyors, Cooke and Reeves trace the development of a large number of individual arroyos. As they acknowledge, the extension of certain Californian arroyos was documented by W. B. Bull (*Am. J. Sci.* **262**, 249–258; 1964). Their own study is much more extensive. It demonstrates that wherever the history of an arroyo can be traced, individual events and acts of interference by man have been responsible for its initiation and enlargement. Cultivation of valley floors and destruction of vegetation along shallow watercourses have usually played an important part; but in the case of the San Simon arroyo, for example, a wagon route seems to have been the immediate cause of gullying, whereas the Santa Cruz arroyo was initiated by water supply channels. Elsewhere, concentration of drainage water by culverts or other man-made features started the downcutting.

Arroyos have destroyed floodplains, accentuated flood peaks and accelerated sedimentation to the detriment of the environment of the American South-West. We now know that they are the outcome of a multitude of individual events and that sweeping explanations are inadequate. Cooke and Reeves have labouriously but effectively sawn through the Gordian knot tied by earlier investigations.

A. T. Grove

A. T. Grove is Lecturer in the Department of Geography at the University of Cambridge, UK.

Stereochemistry

Stereochemistry: An Introductory Programme with Models. By J. Pearce and E. Glynn. Pp. 171+cut-out kit. (Wiley: London and New York, 1976.) £2.75; $5.

TEACHERS involved in imparting the basic principles of stereochemistry in organic molecules to their students are always faced with the problem of persuading the students to purchase sets of molecular models and to use them to build three-dimensional structures, thereby gaining a better understanding of stereochemistry. Pearce and Glynn have produced a kit of models and shapes together with a programmed text containing clear and detailed instructions, so that a student may work at his own pace, in discovering the essential principles of stereochemistry and conformation. A number of fascinating shapes are provided which enable the student to gain an understanding and appreciation of chirality and its importance in stereospecific reactions in both chemical and biochemical systems.

The models are of the well-known 'orbit' type and are easily assembled; cyclohexane and its derivatives in particular are easily constructed and manipulated. The programmed text includes a progressive series of questions for the student to answer, often as a result of the assembly and study of models or shapes. The answer is provided after each question and at the end of each section the essential principles relating to it are reviewed.

The text begins with a discussion of the various types of molecular models, and instructions relating to the construction of models of simple covalent molecules using the kit provided. Conformations of open-chain and ring compounds are discussed, followed by a consideration of chirality and restricted rotation. The various strategies for examining molecular shapes are explained, and the objectives of the learning program summarised. A series of appendices are provided at the end of the text, some of which the reviewer would have found more useful if given either at appropriate places in the text or at the beginning. The text might also have benefited from a more detailed consideration of the D/L and R/S conventions.

This is an excellent programmed text and set of models and shapes for a student to use in discovering the principles of stereochemistry. It is good value for money and can be strongly recommended. A larger set of models attached to the text, however, would be desirable for long-term use to enable the student to build larger models, pertaining to his own interests and possibly outside the range of the text.

D. I. Davies

D. I. Davies is Reader in Organic Chemistry at King's College, University of London, UK.

Aspects of hydrology

Facets of Hydrology. Edited by John Rodda. Pp. xvi+368. (Wiley: London and New York, 1976.) $39; £19.50.

THIS book is a bold attempt to bring together, in a single volume, papers describing much of the hydrological work which has been undertaken in the past decade. Most of the authors are internationally recognised experts, although a few are little known outside their own country.

The stated aim of the book is to provoke the interest of a range of readers, both researchers and practitioners, who are hydrologists or scientists working in allied fields. These aims are achieved with a fair measure of success but, as is often the case with a book produced for a wide and diverse audience, it is not entirely successful. At the one extreme some chapters may be regarded as superficial by experts in the particular subject; at the other there are chapters which are written for readers who themselves already have a very specialised knowledge.

Nevertheless, every reader will find much of interest in a book which will enable him to acquire knowledge of many of the facets of hydrology with which he was not familiar.

It is not possible to comment on each of the chapters in the book and to single out any particular chapter is simply to reflect the reviewer's own interests. The chapters on river gauging, water quality, basin studies, statistical methods, and law, however, each demonstrate in their own way, the diversity of material presented.

In the chapter on river gauging three new methods are discussed with a sensible balance between theory and practical applications. The fact that there is a chapter on water quality will be particularly welcomed by those people who have always argued that the division between water quality and water quantity is an artificial one. The chapter on basin studies presents a representative selection of the very wide range of work undertaken in recent years. That on statistical methods is one of the chapters requiring a fair degree of specialist knowledge. It tackles the problem of spatial variation in hydrological variables in a way which will be extremely useful to those people who are confronted with the problem. The chapter on law is a comprehensive review of the water law and will be of interest to all water resource engineers.

The overall impression is of a book which should find its way on to the shelves of most serious hydrologists and of all well stocked libraries.

M. J. Hamlin

M. J. Hamlin is Professor of Water Engineering at the University of Birmingham, UK.

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Zeolite catalysis

Zeolite Chemistry and Catalysis. (ACS Monograph, 171.) Edited by J. A. Rabo. Pp. viii+796. (American Chemical Society· Washington DC, 1976.) $65.

In the preface to D. W. Breck's *Zeolite Molecular Sieves* (Wiley-Interscience, 1974), the author states that the important subject of zeolite catalysis was deliberately omitted. This serious omission has now been rectified by the publication of the only other major work in this field, edited by Jule Rabo, an associate of Dr Breck at the Union Carbide Corporation. All zeolite chemists now have two texts to keep on hand, which between them cover all the aspects of the subject and survey many important papers published up to the end of 1973.

It is a pity, however, that a book as costly as this one could not have been published more quickly, so that there is not such a wide gap in the literature. The book has, however, been carefully edited. There are only a small number of printing errors, most of which are obvious to a reasonably well-informed reader.

Six of the chapters are written by personnel from the Union Carbide Corporation, making the book somewhat biased in its outlook. This is a pity considering that much important work in this field is being carried out in European and Japanese laboratories. It is also a pity that a chapter emanating from the Mobil laboratories, where some of the most important research in this field has been carried out, could not have been included.

Despite these criticisms, this is an excellent book which will be an essential acquisition for all those working in this field. **L. V. C. Rees**

L. V. C. Rees is Senior Lecturer in Physical Chemistry in the Department of Chemistry at Imperial College, University of London, UK.

Unity of chemistry

Inorganic Aspects of Biological and Organic Chemistry. By R. P. Hanzlik. Pp. xvii+402. (Academic: New York and London, 1976.) $37; £26.25.

Although I agree whole-heartedly with the intentions expressed in the foreword to this book I am somewhat dissatisfied with the way in which they have materialised. It is true that interdisciplinary areas such as those between inorganic and organic chemistry (organometallic chemistry) and between inorganic and biochemistry (bioinorganic chemistry) provide a teaching opportunity which, if properly accepted, could show a student the unity of chemistry. The obvious danger is that, while stressing the interdisciplinary elements of the topic, the clear strengths of the disciplines themselves are lost.

The title tells us that the three disciplines we need are those of Inorganic Chemistry. Organic Chemistry and Biology. The author tackles inorganic chemistry bravely in the introductory chapter and covers the organic chemistry well in later chapters (that is, its inorganic aspects). As there are many good texts on organometallic chemistry, however, I looked for something extra which would provide the essential link with biology. Surely this must be through topics such as oxygen insertion, Grignard reagents, hydrogen activation, ester and amide synthesis, hydrolysis, and so on. I feel then that I should have been given detailed contrasts between peroxidase, cytochrome P-450 and Fenton's reagent; magnesium Grignard reagents and vitamin B_{12}; Wilkinson catalysts and hydrogenases; and between Lewis acid–base catalysts and metalloenzymes Unfortunately many of these topics receive scant treatment. The reason is I believe a lack of insight into biology, and I therefore look upon this volume as a brave and worthy attempt, rather than a great success, for I miss the special features of the discipline which is biology.

Surely the staggering feature of all biological chemistry is organisation without simple order. Even trivial compounds such as calcium carbonate have to be regarded with different eyes when they appear before us in shells, with their enormous variety of shape and form. Again, to mention cytochromes and not the organised system of catalysts of photosynthesis, or to leave out chlorophyll and its special organised function in the leaf, or to omit halogenation by peroxidases and the relation to immune systems, leaves the feeling that the inorganic aspects of *biological* chemistry are not seen in their true depth.

Dr Hanzlik has produced a good book, but there was the material here for a really wonderful one

R. J. P. Williams

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Insect viruses

Virus–Insect Relationships. By Kenneth M. Smith. Pp. viii+291. (Longman: London and New York, 1976.) £11.

A NEW BOOK on insect viruses is a rare and welcome event for those who work in this area, so a first reaction to it is naturally one of enthusiastic interest. This book is a well produced volume, nicely laid out and printed in a clear easily read type. There are many illustrations but most of the electron micrographs are marred by their flat grey tones. It is a pity that closer examination soon tempers this first impression. Some fears are aroused by certain comments in the preface: "This book has been written by one of the few remaining old-fashioned virologists" and "detailed accounts of the chemical nature of the viruses and their molecular biology have been omitted". Is this an apology for an incomplete or outdated treatise?

The book is divided into two parts, one giving an account of the types of virus and virus disease affecting insects, the other discussing more general aspects such as infection and replication. Although there are advantages to such a layout, it does mean that repetition occurs and narrative lacks cohesion. The further division of the chapters in the first part of the book into a general account of the virus group followed by descriptions of specific viruses was ill-advised. Both repetition and contradiction occur as a result. The general account component of each chapter decreases progressively from two and a half pages in chapter 1 to four sentences in chapter 7 so possibly the author by then had appreciated the difficulties that this approach creates. The book has other structural peculiarities. Just one example is the grouping together of parvoviruses and picornaviruses in one chapter (with a short general introductory account dealing with parvoviruses only), whereas viruses of the honey bee and *Drosophila* species are deemed worthy of a separate chapter despite the fact that most of them are small spherical RNA-containing viruses anyway.

The chapters in this first part of the book describe the various groups of insect viruses mainly from a morphological standpoint, with only spasmodic attempts to include relevant biophysical or biochemical data. More recent efforts in insect virus research, such as the definition of methods and criteria for identifying the large number of virus isolates from different insects more reliably than by naming the host insect, are given scant attention. Not only are such recent data omitted but certain accounts also seem outdated conceptually and there are several examples quoted of observations or hypotheses which would not now find great support. There are some errors also. For example, the 1.38% DNA quoted for one insect poxvirus has since been corrected to nearer 5%, and Fig. 5.6 is in fact a picture of *Wiseana cervinata* iridescent virus and not that of *Costelytra zealandica*.

In the second part of the book the chapters on serology, nucleic acids and tissue culture in particular are poorly done. Similarly the account of possible hazards in applying insect viruses as pesticides does not do justice to the amount of time and effort spent on this topic in the last three years. Surprisingly it is only in this chapter that one can find any mention of the epizootiology of insect virus diseases. I am surprised also to find staining methods for optical microscopy included as a separate chapter when little effort has been made to describe laboratory techniques elsewhere. For example, the chapter on insect cell culture gives no details of available cell lines or media (in contrast to the chapter on insect rearing where details of synthetic diets are provided) and the reader is referred to somewhat out-dated sources of information.

Two chapters are included on vectors of viruses. The one on "Insects and other arthropods as vectors of plant viruses" is a useful general account, although many plant virologists will be surprised to read that semi-persistent or circulative viruses show no latent period in the vector and do not survive after a moult. One confusing aspect of this chapter is that its subdivision by headings of different type face and size seems quite illogical. The chapter on "Insects and other arthropods as vectors of animal viruses" is of little value. It is woefully brief for a topic of this magnitude and importance, and the examples described appear to have been chosen at random. Both of these accounts seem rather out of place in the book, encroaching as they do on other major areas of virology, and they should perhaps have been given a separate section at the end.

The impression left after reading this book is that sadly it is an opportunity missed. It is quite uncritical and often strangely unbalanced in that examples of work have been selected for description, and others excluded, apparently without valid reason. Insect virology has become a more challenging and exciting subject in the last five years. The book conveys little of this to the uninformed reader. To the specialist it is a disappointment. But this book has no competitors and therefore it has some value and usefulness simply because it is a modern text on insect viruses. As such it will find a place on library shelves as a teaching aid and a reference source. We deserved better. **K. A. Harrap**

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Iron–sulphur proteins

Iron–Sulfur Proteins. Vol. 3 : Structure and Metabolic Mechanisms Edited by W. Lovenberg. Pp. xiv+443. (Academic : New York and London, 1977.) $39.50; £28.05.

SINCE the publication of volumes 1 and 2 in this definitive series in 1973 a number of significant advances have been made in the study of iron–sulphur proteins. These are reviewed in this volume which again is a 'must' for anyone interested in the biology and chemistry of iron–sulphur proteins.

Beinert's chapter on the iron–sulphur centres of the mitochondrial electron transfer system covers "those developments of the last five years that have made the cytochromes minority constituents". The multiplicity and diversity of iron–sulphur centres makes it necessary to have an electron paramagnetic resonance apparatus to study electron transport in mitochondria. It is a pity that the iron–sulphur centres in photosynthetic membranes were not dealt with similarly.

The enzymology of nitrogenase has advanced significantly and is excellently reviewed by Orme-Johnson. The genetics of the nitrogen-fixing (*nif*) gene and the regulation of these genes is the subject of Valentine's chapter. The transfer of *nif* genes to non-nitrogen-fixing organisms is an exciting possibility for the future.

Mössbauer spectroscopy has been crucial in the understanding of the electron transfer properties of the iron–sulphur centres. Two chapters by Cammack, Dickson and Johnson, and by Debrunner *et al.* review the evidence which has been used to adduce structures of the 1Fe, 2 Fe and 4Fe centres, and help confirm X-ray evidence discussed separately by Carter.

Finally, the chapter by Holm and Ibers on synthetic analogues of the active sites of iron–sulphur proteins describes the status of their work. Holm's group has synthesised analogues of 1Fe, 2Fe and 4Fe centres. The chemical and physical properties of these centres are summarised, and make beautiful reading. **D. O. Hall**

D. O. Hall is Professor of Biology in the School of Biological Sciences at King's College, University of London, UK.

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obituary

W. H. Morton

WILLIAM H. MORTON, for seven years Assistant Professor of Geology at Addis Ababa University, Ethiopia, was shot to death by Government troops in Addis Ababa on 10 March 1977, one of the many victims of the current political disturbances in Ethiopia.

Bill Morton graduated from Manchester University and served his apprenticeship with the Geological Survey of Uganda. A field geologist *par excellence,* he belonged to that select group of British geologists with detailed first-hand knowledge of the remoter parts of East Africa.

In addition to his own research publications, always the fruit of long months of painstaking geological mapping with his colleagues and students, Bill had a very real desire to share his hard-earned geological knowledge with students and teachers throughout the country. He had recently completed a handbook for non-specialists on the rocks, minerals and fossils of Ethiopia, and was awaiting the comments of his colleagues. Its publication may have to await happier times. Of more immediate value to the government of Ethiopia are the geological maps of many parts of the country, including the Addis Ababa region, which Bill and his former students produced with that attention to detail and high degree of intellectual honesty which were amongst his outstanding qualities.

The Afar Triangle has stimulated new concepts in geology which have repercussed far beyond Africa, and Bill Morton made his own contribution to the knowledge of a region that has attracted so many distinguished earth scientists. Starting with a recognition that the tilted fault-blocks bordering the Afar were the result of extension tectonics and not, as previously thought, of folding and decompressive stresses, Bill and his colleagues went on to develop a model of crustal attenuation as part of a continuous process of plate separation. His studies of Afar tectonics, of the age trends of plateau and rift margin volcanics in Ethiopia, and of crustal thinning at rift margins, some of which appeared as seminal papers in *Nature,* were always conveyed in clear and simple prose, a by-product of his experience as a teacher.

A level-headed and thoughtful person, Bill Morton was a tireless teacher, devoted to his students and immensely concerned with imparting practical and worthwhile knowledge. He was equally at home on the summit of a glaciated volcano as at the bottom of Ethiopia's deepest known pot-hole. Well loved by students and colleageus, he was highly respected by geologists from many countries. In a tragic moment, Ethiopia has lost a man who had devoted his career to her scientific advancement and welfare. His many friends and geological colleagues both inside and outside Ethiopia will remember him with love and professional admiration.

D. Adamson, F. M. Dakin and
M. A. J. Williams

Hans-Lukas Teuber

HANS-LUKAS TEUBER, Professor of Psychology at M.I.T., was lost on January 4 while swimming off the British Virgin Islands. It is thought likely that he died of heart disease; he was 60 years old. Many in Britain will share the sense of loss, not only because "Luke" (as he became known in later years as he became fully absorbed into the American culture) was an indefatigable traveller and lecturer, but also because of a memorable year as Eastman Visiting Professor at Oxford in 1971-2. His audience and influence were deservedly world-wide.

In later years he was perhaps best known as the father of a distinguished, indeed unique, centre of psychology and brain sciences at M.I.T., to which his devotion was complete and in which his pride was transparent. But it was his earlier highly penetrating and influential analyses of the problems and mechanisms of visual perception, in particular, and of brain function more generally, with which he first made his mark. His early major researches were on the psychological analysis of brain wounds in war veterans, carried out while serving with the U.S. Navy during World War II. This type of research remained a consuming interest until the end, but he contributed a unique and distinctive personal approach to a tradition that had its roots in 19th century neurology. To that tradition Teuber injected a concern for the importance of certain crucial demands that modern psychological analysis places upon the interpretation of brain function. He was, for example, one of the first to seize upon the importance of the distinction between externally imposed changes in sensory stimulation and those that result from self-initiated movement, which produces a "corollary discharge." He was quick to spot and integrate important modern developments, both technical and intellectual, but these always were seen in a historical context which he viewed with enormous erudition and reverence.

Born in Germany and educated at the French College in Berlin and at the University of Basel, he emigrated to America during the War and received his doctorate in psychology at Harvard in 1947. He claimed that he was accepted as a desirable emigré, when his ship was stopped at Gibraltar, because he was able to converse freely in Latin and Greek with the young inspecting British officer. His father was founder, for the Prussian Academy of Sciences, of a field station for the observation of chimpanzees on Tenerife, whose fame was later assured by the important work of Wolfgang Köhler (*The Mentality of Apes*) carried out there. This perhaps accounts for Teuber's early interest in problems of visual perception identified by the Gestalt psychologists, and his friendship and admiration for Köhler were life-long.

At Harvard, the story is told, he managed to fail the compulsory German translation because, as a new arrival, he did not know enough English into which to translate the German. But, if so, his command of English soon developed to lofty heights, and he could hold an audience rapt with his eloquent and gently humorous style. In 1947 he joined the faculty of the Bellevue Medical Center of New

York University, where he remained until 1961, when he became Professor of Psychology at M.I.T. His quickness of repartee was renowned. On one occasion, when chided at a symposium by an impatient participant for his frequent references to 19th century authors, Teuber replied without hesitation that he merely wanted to display his originality. And he took a quiet delight in his skill at improvisation and extemporaneous delivery. When a lecture to a large audience in a playhouse was interrupted by a power cut, he continued his lecture, without undue pause, by using his fingers in front of the emergency lamp to project his slides in the form of shadows on the screen.

His move to M.I.T. was at first somewhat turbulent, perhaps because Teuber had a clear and even uncompromising conception of the type of department of psychology he wished to develop. To it he attracted scientists of great distinction from a variety of disciplines, as well as younger persons whose promise later was fulfilled; the contributions of his colleagues were as important in neuroanatomy and neurophysiology as in experimental psychology. He worked unceasingly to attract funds for their endeavours and to promote a genuinely interdisciplinary atmosphere, warm and paternalistic, in which he and his colleagues could flourish. The M.I.T. department became an almost compulsory stopping-off point in the U.S.A. for scientists from throughout the world with interests in brain function and psychology; they were invariably greeted with great hospitality and kindness, their seminars almost always continuing at the Teubers' home late into the evening, surrounded by a formidable but enthusiastic circle of graduate students.

Ironically, his memorial service was held at M.I.T. on the day on which he was to have received an award given by the Institute to a member of its faculty for outstanding accomplishment. The citation described him as "a man who joins the instinct of a penetrating experimenter and the sympathetic experience of a true humanist with the consummate style of a gifted teacher." To these qualities may also be added his warmth, skill and dedication as a promoter of neuropsychology both at home and abroad. He was an insightful, popular, and expansive reviewer at international meetings—even as a helpful translator for foreigners, the duration of his commentaries was apt to exceed, by a considerable amount, that occupied by the original speaker. As tragic as was his death, perhaps he would have preferred it to any alternative that might have prevented him from exercising his indefatigable energies at full strength.

He is survived by his wife, Marianne, who was a devoted partner throughout his career, and by two sons, Andreas and Christopher.

A. T. Phillipson

Professor A. T. Phillipson, who died suddenly on 10 January at the age of 66, was the leading figure in the great post-war revival of interest in the function of ruminant animals. He graduated in agriculture at Cambridge, gained his MRCVS at the Royal Veterinary College, London and then returned to Cambridge to show his flair for research in a Ph.D. study of gastric motility in sheep. Subsequently, he led a small wartime group, under Sir Joseph Barcroft, FRS, at the ARC Unit of Animal Physiology, Cambridge. Between 1941 and 1947 Phillipson, with Dr R. A. McAnally, showed that the hitherto neglected volatile fatty acids are the final end products of microbial digestion of carbohydrates and that they are absorbed through the heavily keratinized epithelium of the rumen into the portal blood in quantities which provide a significant proportion of the energy requirements of the host. Such a radical conclusion was not accepted without controversy, yet attempts by others to find an alternative explanation only reinforced the evidence in its favour. In fact in the last thirty years the conclusions made by this small group have formed the basis for much of the subsequent work on ruminant digestion throughout the world and, when they were extended to other components of the diet, such as protein, completely changed our concept of ruminant nutrition and metabolism. It is salutary to remind ourselves that this major advance was based, after a preparatory period in the late 1930's for thought and observation, on a series of simple and elegant experiments carried out over the relatively short period of about four or five years.

Moving in 1947 to the Rowett Research Institute, of which he became Deputy Director in 1952, Phillipson consolidated the advances made at Cambridge. The years at Aberdeen were marked by special attention to the techniques required for the quantitative assessment of the ruminant digestive processes. Techniques ranging from microbiology to radiography were brought to bear on microbial fermentation and synthesis, ruminal absorption and metabolism of volatile fatty acids, movements of the forestomachs and the propulsion of food to the intestine. He was one of the first to emphasise and measure the extent to which urea may be secreted into the rumen to provide a substrate for microbial protein synthesis. In a prophetic mood in 1947 (*Vet. Rec.* **58**(8), 81–85) he had stressed the importance of measuring the amounts of protein and polysaccharide received by the animal in microbia form, of knowing how much starch and protein escapes degradation in the rumen and of assessing the amounts of volatile fatty acids absorbed. In 1977 we are beginning to use such information in evaluating diets.

In addition to his important research, Phillipson wrote a number of lucid reviews outlining the state of knowledge of rumen function, always with exact attributions and a minimum of extrapolation, and pointing the way to further essential investigation. These were required reading for a new generation of animal scientists and must have directed many to the fascination of the rumen. A remarkably high proportion of the active post-war workers in this field spent time with Phillipson, some to experiment, some just to discuss, but all to be welcomed, involved in his interests and influenced for the future. He travelled a good deal and those he met were also much influenced. In such benign ways, Andrew Phillipson has left a permanent mark on rumen studies. His hallmarks were enthusiasm, wide background knowledge, a preference for direct approaches, accuracy and a habit of being right.

Returning to Cambridge in 1963, Phillipson took the Chair of Veterinary Clinical Studies. Administration and teaching then restricted his opportunities for research, but in these activities he preserved the high standards characteristic of his earlier work. He was chairman of the organising committee of the very successful Third International Symposium on Physiology of Digestion and Metabolism in the Ruminant at Cambridge in 1969.

Phillipson was made FRSE in 1953 and his achievements were recognised by honorary degrees at Copenhagen, Ghent and Edinburgh. In England he received major prizes in agricultural and veterinary fields.

Andrew Phillipson was a charming man whose enthusiasm shone through a diffident manner; an aesthetic, person, especially fond of music, he enjoyed Cambridge and his Fellowship to the full. His family life was extremely happy and the sympathy of many friends, worldwide, will go to Mrs Rachel Phillipson and their three sons.

C. C. Balch

nature

7 April 1977

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Will the JET age ever come?

THERE is only one thing more frustrating than the well-known impossibility of learning precisely when particular European Community decisions are made. That is learning that they are not made. Last week's debacle at the Energy and Research Council meetings in Brussels was nothing if not frustrating.

Two major issues, among many others, confronted the Energy Ministers: the minimum safeguard price (msp) for oil, to protect investment in the North Sea and in alternative energies; and implementation of an agreed Euratom loans scheme for construction of nuclear power stations. The two have always been linked, with Britain refusing to sanction implementation of the loans scheme without agreement on the msp, and France preferring selective support to the msp.

Only one issue, or so it seemed, confronted the Research Ministers: where to site JET, the Community's fusion project. On that decision also hung the implementation of the four-year programme of the EEC's Joint Research Centre (JRC), agreed in principle months before. Since the JRC establishment at Ispra stood to benefit by the programme, its candidacy for JET had weakened next to Culham in Britain and Garching in West Germany, but France had pushed intensively for Cadarache.

With one European Commissioner (Guido Brunner) now handling both the Energy and Research portfolios, and growing talk of JET as an energy rather than as a research project, the idea of agreement in one council permitting agreement in the other emerged as soon as the two council meetings were set for the same day. Sure enough, progress in the Energy Council produced British concessions on the msp, and alternative schemes will now be examined. Implementation of the Euratom loans scheme was then agreed, and the hope was accordingly expressed that this would assist the Research Council.

It didn't. The Research Council could not agree on a site for JET, for the new but genuine enough reason expressed by France that critical organisational and financial details concerning the way the project would be run were not finalised. What is astonishing is that these matters had not been agreed in the interminable meetings held at all levels over the previous 18 months. What is surprising is that the Council nodded through the four-year JRC programme anyway.

Apart from the embarrassment (especially noticeable in Britain) that goes with such developments, the result is that a decision on JET is no longer contingent upon a tangled web of linked issues. That may mean an early decision if the new difficulties can be clarified, but no one is counting on it. Crazily, the only action has again been inaction.

Though this is an era of international competition and energy crisis, our worry is not whether or when a breakthrough on fusion comes, and certainly not who achieves it. Science does transcend frontiers. Our worry is the uncertainty which indecision generates. JET is an experiment, not a reactor, and its research team deserves better. Now may be an appropriate time to examine the thinking which makes international scientific projects matters of national prestige. Otherwise the Community's processes, though favouring compromise and calculated not to alienate, run the risk of doing the opposite by too faithfully serving governments rather than people. ☐

Science as a profession

THE founding fathers of the Royal Institute of Chemistry, which was last week celebrating its centenary, envisaged a body which would act for chemists rather as the Royal Colleges do for medical men or as the Inns of Court do for lawyers. In spite of a distinguished record in many respects, the Institute has in that aim been totally unsuccessful Neither chemists, nor indeed any group of professional scientists, form a comparable profession. Scientists and engineers are neither as secure nor as confident nor as well remunerated as doctors or lawyers. Society may view this favourably; scientists find it uncomfortable.

The difference must in part be because most scientists are neither self-employed nor fee-earning, and may consequently find themselves out of work in a recession. Sir Harold Wilson, in praising chemists at the centenary celebrations, cited the case of his own father, a dye chemist, who became Winston Churchill's election agent while out of work for two years—perhaps persuading the infant Wilson of the surer future available in Parliament. Yet, although not as secure as members of the other liberal professions, professional scientists are expected to have similar standards of ethics and behaviour. In addition to integrity in research and loyalty to an employer, particularly as regards confidentiality, scientists are expected to speak out if their work throws up any environmental or health hazards, even though such revelations may damage their employing company.

Some of the disquiet scientists feel towards government was expressed last week by the President of the Royal Institute of Chemistry when, in reply to the former Prime Minister, he voiced the irritation at the currently fashionable expression "the two sides of industry". The point is, research scientists in industrial laboratories do not see themselves as being on either side. They feel their views are not being sought, much less heard or heeded, on major issues. When British opinion is being moulded to support wealth creation, they believe that as major wealth creators they are not beneficiaries of that support.

Crucially, scientists see the innovations of research and development as the source of profit of leading companies—failures to exploit science (as with the loss of patents on antibiotics or jet engines) not being the fault of the originators. Chemists in particular are disgruntled because they feel largely responsible for the country's thriving chemical industry and its near relatives in petrochemicals, pharmaceuticals and so on. The companies concerned do not include any of the famous lame ducks. They are almost never involved in industrial disputes. They are highly scientifically innovative. They make the profits which are taxed to support other industries unable to stand up to international competition because, in many cases, of their small research investment.

In spite of this scientific and commercial success, the people who carry the burden in research do not see themselves in a secure profession. Both output of and demand for chemists has declined during this decade and there is evidence that the quality of the newly qualified has dropped. The medical and legal professions, on the other hand, control both entry and the right to practice as well as the discipline of their professions, thus evading the cyclical imbalance in supply and demand so often felt by scientists.

Science as a profession is in a weak position. The best scientists are evenly spread over the country and not concentrated in the capital with day to day access to Whitehall. Salary structures are strongly influenced by central government because the latter is the major employer, basing its rewards on comparability with industry which in turn compares with civil service salaries. It is a small but vicious circle which depresses salaries.

If the recent Bullock proposals for worker-directors in Britain are implemented along anything like the lines suggested in the majority report, the new directors are unlikely to include research scientists. Yet scientists are a major wealth-creating source. They may not continue to provide for the community if their views are not sought or are disregarded, and certainly if they are driven overseas by the tax structure. ☐

A parable on recombination

DeWitt Stetten offers some personal thoughts on the management of hazards in recombinant DNA research

SCIENTISTS and nonscientists are currently polarised in their assessments of the hazards attached to the creation and handling of recombinant DNA molecules. Whereas the hazards are of unknown dimensions, the anxieties are clearly great: but the magnitude of the anxiety is a very imperfect measure of the magnitude of the hazard. Among the diverse views we are seeking an area of possible agreement or, better yet, an area of truth.

Truth is sometimes found in unexpected places. It may be recalled that Jonathan Swift, famed satirist and author of *Gulliver's Travels,* while serving as Dean of St Patrick's in Dublin, considered the disastrous problem of the famine among the Irish peasants which was aggravated by the high birth rate and the abundance of children. He analysed the problem in 1729 in an essay entitled 'Modest Proposal for Preventing the Children of Poor People from being a Burden to their Parents or the Country,' in which, with all pretense of reasonableness, he concluded that the entire problem could be solved if the people of Ireland ate the children. In view of the evident success of Swift's satire, I am taking the liberty of presenting here a parable which I call 'Genetic Recombination—A Modest Proposal'.

After all, recombinant DNA technology is a special case of a far more general phenomenon—that of genetic recombination itself, where genetic material from two sources is repeatedly combined within a single cell. It happens when an oncogenic virus invades a mammalian cell, or when a bacteriophage invades a bacterium. It happens when two bacteria enter into conjugation and transfer plasmid DNA from one to another. In the laboratory it can be achieved through the hybridisation of eukaryote cells from widely differing species, and it is of course the very basis of sexual reproduction.

A form of this kind of experimentation which has been popular for a very long time indeed results in the fertilisation of the human ovum. I refer to this exercise as an experiment, because it always is experimental in that the outcome is not known *a priori.* At this time we can not even foretell the sex of the offspring, much less any of the details of its anatomy or character. Yet the experiment, whereas it provides great possibilities of useful and benevolent results, does have a real hazard. Textbooks of obstetrics contain many engrossing photographs of anatomical monsters which have resulted from this process, but it seems to me that the very worst monsters, the behavioural ones, are not included. These may be divided into two classes—the societal monsters such as gangsters, typified by Al Capone, and the international monsters, exemplified by Adolf Hitler.

Clearly, some of these monsters possess a survival advantage over the rest of us. One has merely to note the direction in which the machine gun which the gangster carries is pointed to ascertain which party is in the better position to survive the encounter. Of particular interest is the experiment conducted nearly ninety years ago by the parents of Adolf Hitler. The maternal ovum may be construed in this case as the host, while the paternal sperm clearly was the vector, bearing as it did a charge of DNA which was foreign to the host. The conceptus which resulted was carefully cloned under reasonably sterile and controlled conditions, and there arose an individual who became, directly or indirectly, responsible for the premature deaths of 4×10^7 human beings in the course of about eight years. This mortality is certainly large, even in the parlance of the epidemiologist.

When scientists first began to appreciate the danger attached to the fertilisation of human ova, they directed attention to the matter and called for an immediate moratorium Since the hazard seemed to be of enormous proportions, the federal government soon became involved; and by and by, a Presidential commission was established to consider the problem and to determine its best resolution. With the moratorium still in effect, one alternative solution proposed was to sterilise all adults, male and female. It was forecast that this procedure would probably work, but it would be quite costly. For this reason, the General Accounting Office, which has the responsibility of surveying costly federal projects, gave its attention to the matter and pointed out that sterilising of either the male or the female population would probably achieve the same desired effect at half the cost. To decide whether to sterilise all males or all females, a special panel of the Equal Employment Opportunity Commission was convened which, with Solomonic wisdom, recommended that since there were approximately equal numbers of males and females in the population at risk, it would appear appropriate to sterilise half of the males and half of the females.

A small experiment was conducted on normal volunteers to test the effectiveness of this procedure, and it was found, for reasons which are still unclear, that this procedure would not yield the desired result. After considerable deliberation, the commission recommended that experiments in human impregnation might proceed with caution in certain designated centres, provided steps were taken to preclude pollution of the environment. It was determined that experiments could be conducted under P4 physical containment with all the manipulations to be carried out in class III safety cabinets which, in the usual fashion, would have their entry and exit ports protected either by autoclaves or by incinerators. Only in this way could the public be assured that no monsters would escape to pollute the environment and damage the ecology. The econiche which we so comfortably occupy would not be threatened by another generation which predictably would perform less nobly than we had done. ☐

NASA's Space Shuttle

'Re-usability' is the catchword

Having generated plenty of controversy, the Shuttle is now being proven. **Colin Norman** reports

IF you have a piece of hardware weighing up to 65,000 pounds that you would like to put into Earth orbit after 1980, the National Aeronautics and Space Administration (NASA) would be only too pleased to rent you the Space Shuttle for $20 million. Or if your requirements are more modest —a 200-pound experimental package which doesn't need special handling, for example—NASA would be glad to take it aloft for as little as $3,000. Although some advance notice and a small deposit would be required, it shouldn't be too difficult to make a booking because there is likely to be considerable spare room in the Shuttle when it eventually flies.

With three years to go before the Shuttle is scheduled to begin full service, NASA is starting to drum up business. A list of fees to be charged to such users as the Department of Defense, foreign governments, commercial operations, and individual researchers has recently been drawn up, and NASA officials have developed an ambitious flight plan calling for as many as 60 shuttle launches a year in the late 1980s.

The flight plan indicates that NASA has not changed its basic thinking on the Shuttle since it won approval for the programme after a bitter fight in the early 1970s. NASA officials are still confident, in short, that the Shuttle will revolutionise space activities by sharply reducing the cost of launching payloads and providing routine access to space. If the flight plan is remotely accurate, in fact, it would represent a staggering increase in the space programme, resulting in more activity than during the height of the Apollo era. Many of those who fought the Shuttle programme in its early stages, including some prominent space scientists, remain sceptical of NASA's projections, however.

The design of the Shuttle was determined in 1972, following an intense struggle between NASA and the Office of Management and Budget. NASA had originally wanted to build a fully re-usable system, consisting of an orbiter and a booster rocket, both of which would be piloted back to Earth and re-used, but in the end it had to settle for an economy model which will be only partially re-usable. It will consist of an Orbiter mounted atop an external fuel tank and strapped to two booster rockets. Soon after launch, the boosters will separate and be parachuted into the ocean for recovery, refurbishing and re-use. The external fuel tank will be discarded in the upper atmosphere where it will burn up, and the Orbiter, after completing its mission, will re-enter the atmosphere and glide to a landing like an aircraft.

Launches and landings will take place at two locations, Cape Canaveral in Florida and Vandenberg air force base in California. When launched due east from Canaveral, the Shuttle will be capable of delivering 65,000 pounds into a 185 km orbit, or 25,000 pounds into a 500 km orbit at 55 degrees. Missions requiring polar orbits will be launched from Vandenberg because such launches from Canaveral would involve flying over heavily populated areas soon after launch. The Shuttle can deliver 32,000 pounds into a 185 km polar orbit.

The Shuttle itself is capable of reaching only low Earth orbits, and thus an extra piece of gadgetry is needed to hoist communications satellites into higher orbits and to fire planetary spacecraft out of the Earth's gravitational field. Again, NASA was originally planning to develop a re-usable rocket, called a 'Space Tug', for those tasks, but it has had to settle for a cheaper, disposable system, at least for the time being. It consists of a solid-fuelled rocket, called the Interim Upper Stage (IUS), which will be launched from a Shuttle in orbit; the IUS is being developed by the Air Force. Though NASA officials claim that they still hope to build the Space Tug someday, there is some doubt about whether that day will ever come.

Costs go up

When the Office of Management and Budget finally approved NASA's plan in 1972, the estimated cost of developing the Shuttle was about $5,200 million. The latest projections put the total research and development costs at about $6,800 million, of which about $4,500 million will have been spent by the end of this year. Virtually all the increase is accounted for by inflation. In addition, NASA reckons that it will cost about $2,500 million to produce five orbiters and associated boosters and fuel tanks, bringing the total research, development and production costs to some $9,300 million.

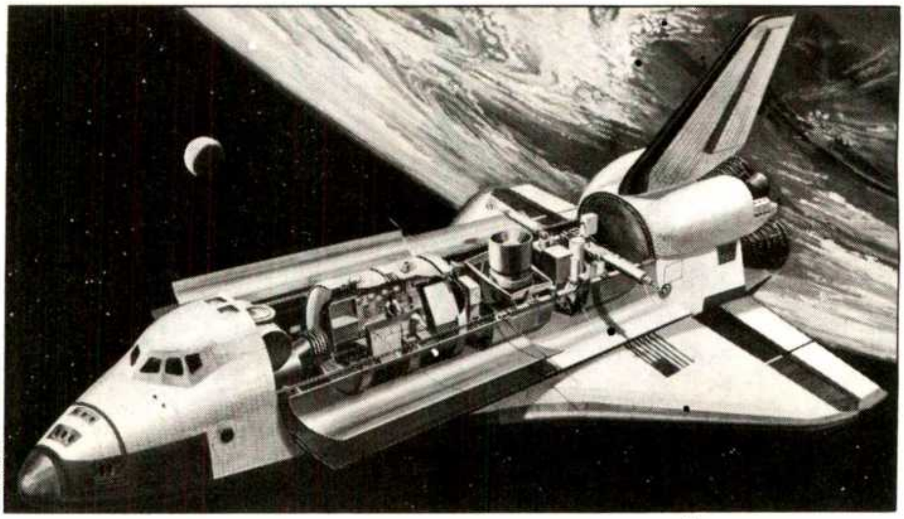
NASA

Shuttle: take-off to recovery

So far, development of the Shuttle is proceeding on schedule and no major snags have been encountered, which is an impressive record for such a mammoth undertaking. The first Orbiter was unveiled last September and, thanks to a massive mail campaign from *Star Trek* fans, it was named the Enterprise. It is now being flight tested in the atmosphere, riding piggyback on a Boeing 747, and in July or August it will be cast loose at 22,000 feet to glide on its own to a landing at Canaveral. The first orbital test flight is scheduled for March 1979, and if all goes according to plan, it will be ready for full operation in the spring of 1980. Development of the IUS is also proceeding apace, and it is also supposed to be ready by 1980.

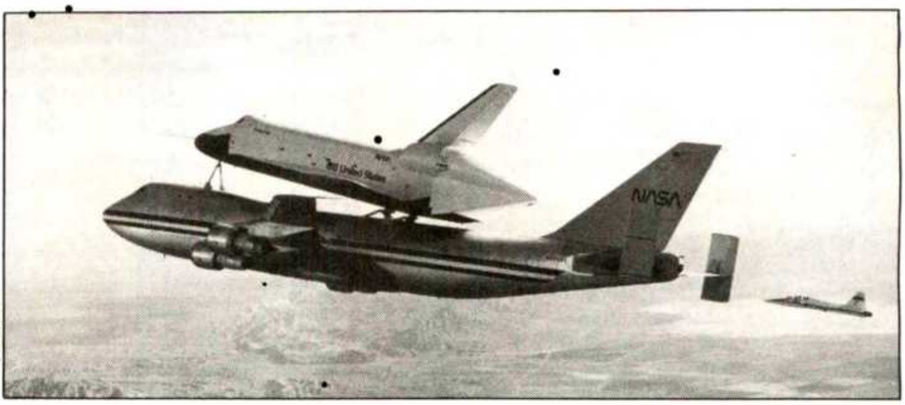
After the Office of Management and Budget gave its approval to the Shuttle in 1972, the real battle began. NASA's cost estimates and projected savings for the system were subjected to severe criticism by a group of Senators led by Walter Mondale, now Vice President, who took their cues from a number of prominent space scientists and systems analysts. They included Thomas Gold, Professor of Astrophysics at Cornell, James van Allen, Professor of Physics at the University of Iowa, George Rathjens, Professor of Political Science at MIT, and Brian O'Leary, a former astronaut.

The nub of the debate was this. NASA claimed that the Shuttle would more than pay for itself over its projected 12-year lifetime because it would be able to deliver payloads into orbit much cheaper than expendable rockets could. Critics of the system argued, however, that NASA was assuming an unrealistically high launch rate with the Shuttle, and that the cost comparisons didn't properly take into account the research and development costs of the system. Another objection was that the costs of developing and building the Shuttle would soak up so



NASA

The Shuttle's Spacelab configuration



NASA

Shuttle takes a trip, March 1977

much of NASA's budget that many space science and applications projects would get squeezed out. The debate raged for about a year, but died down when Congress finally sided with NASA and voted the money to begin developing the system.

At least one of the critics' complaints has already proven correct. While NASA's budget has remained approximately constant in real terms since 1971, spending on the Shuttle has climbed from less than $100 million to more than $1,300 million a year. As a result, there have been very few new space science projects started: some have been scrapped (such as the grand tour of the outer planets), others (such as the High Energy Astronomy Observatory) have been pared, and still others (such as the Large Space Telescope) have been deferred. As O'Leary noted last week, "Our dire scenario has been played out".

Nevertheless, since the project got under way in earnest, NASA officials have been working out a flight plan for the Shuttle based on an assumption that NASA's budget will stay roughly constant in terms of purchasing power over the next 10–15 years, and they have come up with a flight plan very similar to the one which was used to justify the Shuttle in the first place. It should be noted that the plan doesn't represent firm commitments to develop or build a set number of satellites; it is simply a planning document.

NASA calculates that there will be about 560 separate Shuttle flights between 1980 and 1991, with an average of about 60 flights a year between 1985 and 1991. With the massive payload capability of the Shuttle, that represents a very large increase in space activities. According to the flight plan, the breakdown between users will be NASA, 267; other US government agencies, 28; US commercial operations, 60; foreign organisations, 65; Department of Defense, 109. When broken down by type of mission, the plan calls for 229 Spacelab flights, 35 launches of large structures, 168 launches involving upper stages, and 97 launches of free-flying satellites.

Defense's satellites

Aside from the very considerable size of the programme represented by the flight plan, the number of launches envisaged by the Department of Defense is interesting. Though the nature of the defence missions is classified, some two-thirds of them would require use of upper stages, indicating that they are communications satellites or other satellites in geosynchronous orbits. It has also been speculated that the Shuttle's ability to manoeuvre in space will give it the capability of studying, or even capturing, enemy satellites in low orbits.

As for the fees to be charged to users, NASA has recently circulated for comments a proposal which would allow NASA to recoup the operating costs for the Shuttle. The fees would not, however, include payment for a share of the research, development and production costs. Briefly, NASA has proposed that a fee of $20 million would be charged for chartering the entire Shuttle for a single launch. The Department of Defense and foreign governments which have contributed to the Shuttle development would get a discount, as would US commercial operations. Users who require only a part of the Shuttle capacity would pay on a pro-rated basis with the smallest experimental packages which require no manual attention getting in for only $3,000 apiece.

NASA reckons that those fees represent about a 40% discount in launch costs when compared with the use of expandable rockets. Asked whether commercial organisations are showing any interest in booking space on the Shuttle, one NASA official said that he has noticed a recent awakening of interest on the part of payload manufacturers. "At one time they were between lukewarm and disinterested in the Shuttle", he said, "but I have seen a definite reversal in attitude".

As for space scientists, the Shuttle seems to have generated some resentment because of the fact that its development squeezed out a number of scientific missions. Nevertheless, several former critics of the Shuttle contacted last week all agreed that, with nearly $500 million already spent it would be folly to seek to cancel the programme at this stage. And the Space Science Board of the National Academy of Sciences said in a report published in 1974 that the Shuttle "can be an important asset to scientific research in and beyond the 1980s".

At least one former critic of the Shuttle has also recently been converted into a supporter. Brian O'Leary said last week that although he still believes that NASA's justification of the project is seriously flawed, the Shuttle "by good luck" would be a useful transport vehicle for the possible establishment of space manufacturing systems using lunar materials, to construct space habitats and solar power stations. O'Leary, who is now working with Dr Gerard O'Neill at Princeton exploring the concept of space habitats, said that he now supports the Shuttle for that reason. ☐

ESA's Spacelab

Research is the watchword

Spacelab offers scientists unique conditions for future research. **Stuart Sharrock** describes how

THE pioneer phase of manned spaceflight is now over and plans are well advanced for the exploitation era due to begin in 1980. NASA's Space Shuttle will reduce transportation costs and expand the potential applications of space technology by removing many of the constraints imposed by conventional launch vehicles. Spacelab is intended to capitalise on these principles.

A re-usable manned space laboratory with comparatively low operating costs for applications-oriented and scientific experiments, Spacelab is being developed by the European Space Agency (ESA) as a joint European endeavour and will be launched into near-earth orbit in the cargo bay of the Orbiter section of the Shuttle. Thus Europe is for the first time embarking on a new phase of space technology simultaneously with the United States.

The emphasis of the ESA's programme is on satellites for telecommunications, meteorology, Earth resources surveying and air and maritime traffic control. Scientific research is an important but by no means dominant component; the scientific satellite programme concentrates on upper atmosphere and magnetosphere research and astronomy, particularly in the X-ray and γ-ray regions. Spacelab complements these programmes to provide a balanced package of scientific research, but though maintaining the balance, not all future projects under consideration by ESA are likely to be funded. Areas of research will inevitably suffer as the emphasis shifts towards usage of major facilities, but the dwindling national programmes in Europe might be steered to help cover the gaps.

Where Spacelab fits

The Orbiter section of the Shuttle can be launched into near-Earth orbits of various inclinations with an altitude range of 200 to 900 km and a flight time of from 7 to 30 days. Equipment may be placed in orbit or returned for servicing and evaluation after the Orbiter has returned by landing like a conventional aeroplane. The Shuttle concept can be used for launching and servicing satellites, but the accessible range of orbits is limited and conventional 'throw away' launch vehicles will remain necessary unless the inter-orbit 'Space Tug' under consideration by NASA becomes a reality.

Spacelab is a particular unit that slots into the cargo bay of the Orbiter. It is of modular design with two basic building blocks: a pressurised module providing a 'shirt-sleeved' environment for a manned laboratory connected to the living quarters of the Shuttle by a crawl-tube; and an unpressurised pallet on which instruments can be mounted and exposed directly to space. Various combinations of module and pallet are possible, providing a versatile manned space laboratory with a typical experiment payload of 5 tonnes. Up to four instrument operators can be carried (two are planned for the first flight); they will need a scientific background but do not have to submit to astronaut training—the standards required will be similar to those of commercial airline flight engineers.

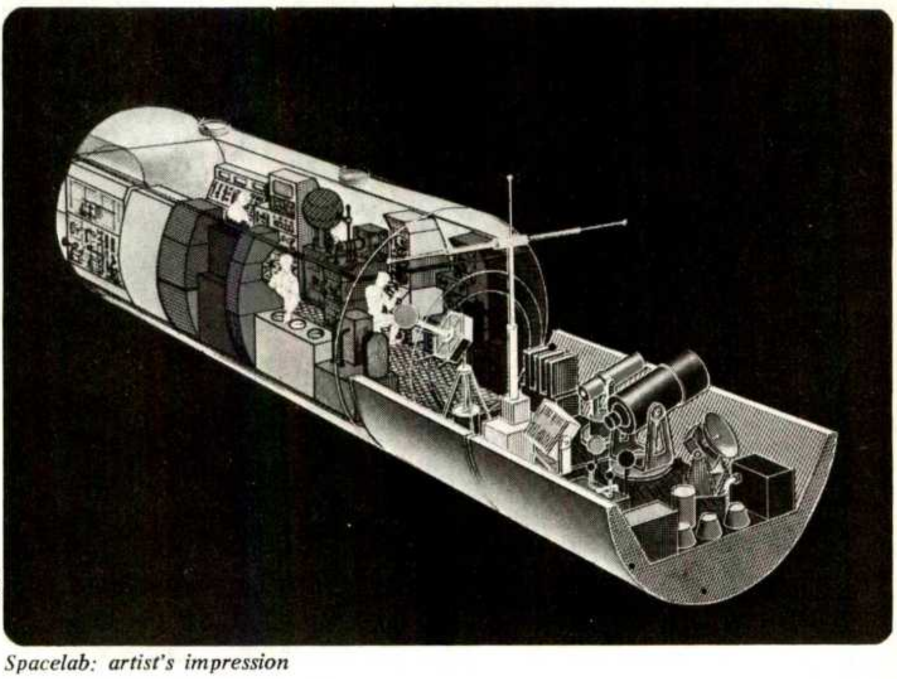
The immediate advantages of this arrangement are that the stringent requirements on weight and volume that bedevil satellite experiments are relaxed, although the problems are to some extent transferred to the strict safety regulations necessary to protect the instrument operators. Existing requirements based on astronaut protection are unnecessarily severe for Spacelab, though some amelioration remains possible. Previous manned spaceflights which have shown the advantages of having operators for some classes of experiments have mainly involved man either as an experimental subject or in his capacity as a dexterous machine; the decision-making capabilities of man in connection with an experiment have yet to be fully exploited. Re-usability is an important new facility: equipment can then be flown many times rather than abandoned at the end of each mission. Perhaps of greater significance is the ability to return materials processed or separated in a space environment and the means of collecting large volumes of data, stored for example on film, beyond the capability of feasible data transmission systems.

Cost immense

Clearly the cost of such an enterprise is immense; the design, development and delivery of one engineering model and one Spacelab flight unit is estimated at around $500 million at mid-1975 prices. Currently the cost of the Spacelab project is under review and, according to ESA, "severe cuts in the deliverable hardware will be necessary in order to accomplish the development project within the established funding ceiling"—not an unusual problem in these times of high inflation.

Value for money is possible if frequent use is made of the facilities. Spacelab is intended to be carried on around 40% of the Shuttle flights and is designed for a minimum of 50 seven-day flights, or about 10 years of operation. This schedule allows the lead time for experiments to be much reduced from the present position with satellite projects. The advantages are clear; the realisation depends largely on funding and management.

Such a large international venture can easily become constipated by its own administration. The Spacelab concept can only be a success if flexibility is maintained for much longer than in



ESA

Spacelab: artist's impression

earlier space projects, but the organisational and management structure have at the same time to cope with an unprecedented mixture of political, financial, technical and scheduling constraints. Major efforts are being made to provide a structure capable of reacting sensibly to these problems.

Experience from previous manned space missions has been invaluable. It is generally agreed, for example, that insufficient thought and preparation went into many experiments conducted aboard Skylab, and a far greater degree of control is being exerted over Spacelab proposals. A simulated Spacelab mission was conducted in 1975 to test out the role of the instrument operators on board Spacelab and the suitability of the proposed management procedures. Short lead times for experiments were demonstrated to be practical, results being obtained from experiments executed only 16 months after proposals were requested, but the mission highlighted the need for greater integration of the instrument operators with the evolution of experiments, and a second more realistic simulation mission is planned for May 1977.

Spacelab research

Areas of research in which Spacelab will provide a valuable addition to satellite facilities include astronomy, atmospheric and solar physics and earth observation. Long continuous periods of observation are often required in these fields and Spacelab is seen as an intermediate step towards mannable orbital systems containing heavy and complicated astronomical equipment that would require regular maintenance; these 'Free-Flyers' would be serviced via the Shuttle.

The use of Spacelab simultaneously for astronomical and Earth observations clearly presents problems, and future Spacelab flights will be dedicated to specific areas of research. The flight profile is not really suited to investigation into the magnetosphere and certain atmospheric phenomena, and in these fields of research Spacelab will be used primarily as a satellite launcher, particularly as the Shuttle is liable to pollute the environment. Measurement of this pollution in terms of emitted gases and magnetic fields is one of the primary aims of the initial Spacelab flight.

For the life sciences and materials science Spacelab will provide a new opportunity for the study of fundamental problems in an environment unattainable on Earth. Additional benefits for the life sciences are the availability of a wide spectrum of high energy particle radiations, whereas materials science and plasma physics welcome the ready attainment of a vacuum over large volumes and the possibility of studying processes under containerless conditions.

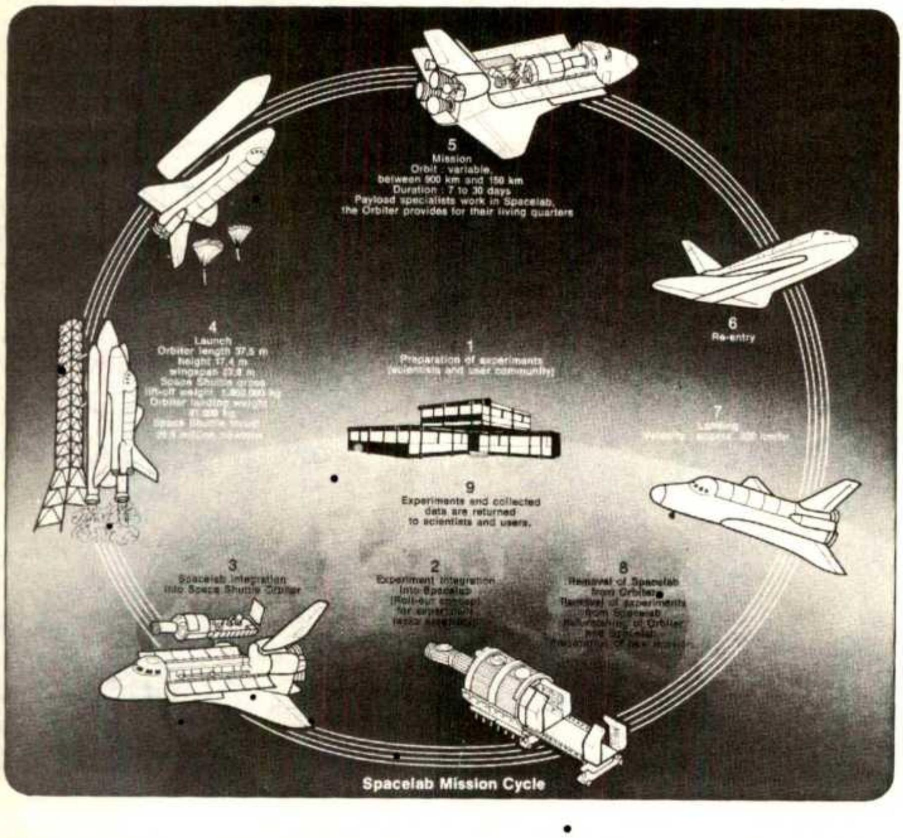
The effects of weightlessness and radiation on biological systems are the major areas of interest in biomedicine on Spacelab, and the first payload will include a Space Sled facility for studying the effects of acceleration on astronauts under weightless conditions. Materials science experiments require steady conditions which preclude the use of the Sled and spacecraft manoeuvres—luckily the majority of these experiments are of short duration.

Commercially the field of materials science offers immense potential and this is reflected in the set of experiments recently chosen as the payload for the first Spacelab flight where materials science experiments outnumber those in all other disciplines. The effective absence of gravity ($<10^{-4}$ g) removes convection and buoyancy effects, so enabling fundamental physical and chemical processes to be studied in the absence of otherwise maskings phenomena and allowing the production of novel and improved materials.

In ESA jargon Spacelab is a 'Special Project'—a programme in which participation by all member states is not mandatory. Countries participating in the finance of the project get a proportionate share of the contracts and use of experimental facilities. The major contributors to Spacelab are Germany (53%), Italy (18%) and France (10%). The initial Spacelab flight in mid-1980 is a joint ESA/NASA mission in which the available facilities are shared equally between Europe and America. The primary objective of the first flight is to demonstrate the capability of Spacelab for space research and to check out all the support systems. Experiments therefore receive second priority, although 100 man-hours have been allocated to them and the necessary test programme restricts the power available to experiments to around 30% of that ultimately to be provided.

In spite of the limited experimental facilities available on the first test flight, the response to the call for experimental proposals has been high. Of over 2,000 applicants, a total of 222 have been accepted, providing 77 experiments from 16 countries. Some proposals were no doubt motivated by the feeling that previous Spacelab experience will carry weight in proposals for future flights; others sprang from uncertainties concerning the funding of these future flights, although these fears will be groundless if Spacelab can be used with its planned frequency.

NASA will charge around $10 million to launch each subsequent Spacelab mission. Details of how this cost will be distributed between participating states within ESA and the experimenters have yet to be worked out. This cost is small compared with the investment, and it will be a pity if details over funding jeopardise the exploitation of Spacelab. It will be a disaster if ESA's future proposals for a balanced scientific programme are affected. ☐



Spacelab Mission Cycle

ESA

USSR

● The need for a closer connection between research and the requirements of the national economy is a constant theme in Soviet planning. Last autumn, the Far Eastern Science Centre was criticised by the Party for having failed to direct its research towards local needs. More recently, the prestigious Siberian Branch of the Academy of Sciences of the USSR came under similar censure. A statement from the Central Committee of the Party, while praising the Siberian Branch for its work in fundamental research, finds "insufficiencies and unresolved problems" in its activity; work on "the complex use of the natural wealth of the eastern regions of the country" is going too slowly; the Presidium of the Branch is paying too little attention to the work of certain centres; and filials, scientific forces and material resources are not being concentrated on "the most important lines of science connected with the acceleration of scientific and technical progress". In particular, insufficient attention has been paid to the problems of the Kansk–Achinsk coal basin, the Norilsk mining and metallurgical combine, and the route of the Baikal–Amur Mainline (BAM) railway.

Instead of admitting these faults and promising amendment, however, Academician Gurii Marchuk, the Chairman of the Siberian Branch, issued a statement extolling the work of the Branch, and softening the rebuke of the Central Committee to a gentle directive showing "ways of improving the quality and efficiency of scientific research". Marchuk stressed that during the 20 years of the Branch's existence, science centres, in addition to its main centre at Novosibirsk, have been established in many cities including Irkutsk, Ulan-Ude, and Krasnoyarsk. The Siberian Branch now includes 48 research and design organisations, representing practically every field of the natural and social sciences. Marchuk himself envisaged the future of Siberian science as "a growing effective force, transforming huge tracts of land along the Leninist path and exerting an increasing influence on many sides of the life of Soviet society".

Within a week, the critical tone of the Central Committee had been de-emphasised even further. Addressing the Moscow meeting of the heads of Academies of Science of the Comecon block, Mr Brezhnev held up the Siberian Branch and the Ukrainian Academy as examples for emulation. "The Siberian Branch is carrying out a very interesting experiment", he said. "The Institutes of this Branch are directly connected to many sections of the national economy, with many major [industrial] enterprises. They have worked out long-term programmes of scientific–technical cooperation and are gradually implementing them."

Since the Brezhnev speech, articles have appeared in the Central press commending the work of individual Institutes of the Branch in the service of the economy—the Hydrodynamics Institute, has been commended for surveying the line for the BAM and the Institute of Atmospheric Optics for weather-forecasting. Marchuk's defence of his Branch seems to have been successful and its present shortcomings have been accepted as temporary deficiencies rather than a failure to understand the fundamental task of the Branch.

● Although the Soviet lunar research programme has so far been confined to unmanned probes and lunokhod rovers, biophysicists at the Siberian Branch of the Academy of Sciences are already working on the possibility of farming in lunar conditions. Experiments with wheat, carrot, beetroot and turnip seedlings suggest that plants could be grown under suitable transparent domes using the natural lunar illumination cycle. The plants were kept at a temperature of 3–4 °C during the 'night' periods, and were grown hydroponically, using common tap water as the nutrient medium. In spite of the lack of minerals, especially nitrogen, and th [illegible]tic illumination cycle, all the plants produced roots with the "normal biochemical composition", and during the second lunar 'day' the wheat produced ears. The team stresses that if plants can be grown successfully by natural sunlight, the energy problem of settlements on the moon would be much easier.

● The current difficulties with international fishing limits have evoked considerable rethinking of Soviet fisheries policy; in contrast to the old theory of large catches and the overfulfilment of targets, the new line stresses that since catches are limited, the entire quota must be suitable for food. The new policy is to be implemented in various ways: Latvia is to use refrigerated factory ships in the Baltic to reduce wastage; and the Deep Sea Fishing Flotilla, operating in the Sea of Okhotsk, has been instructed not to catch Okhotsk grenadier (which accounts for up to 50% of all catches in the area) since the experts have decided that grenadier is unmarketable.

Following a recent order from the Ministry of Fisheries, likely lakes and reservoirs for fish-farming are being appraised, and existing fish-farms are being extended. An experimental trout-farm is already in operation in the out-fall of the Kola atomic power station in the Arctic. The use of fish wastes is also being investigated. According to an official of the Sevryba (Northern Fisheries) organisation, the fishing industry has for some time been able to collect and deliver heads, tails, and gut to shore enterprises for further processing. These waste products, he stressed, contain 30% fat, 8–12% protein and a "considerable quantity" of the B vitamins. At present such wastes are used for animal food, but research is underway on their possible use for human consumption—a project more likely to appeal to the planners than to the average consumer.

● The RATAN-600 radio-telescope, the largest in the world, is at last officially in operation. The telescope, sited at Zelenchuk in the Stavropol' district, was constructed jointly by the Academy of Sciences of the USSR, Moscow University, the Ministry of Power and Electrification, and the Ministry of Power Plant Construction. Mr Brezhnev's formal message of congratulation was issued on 20 March, 1977.

Each of the four sections of the telescope can operate autonomously and part of the Ratan-600 has been receiving signals since 1974. A number of research projects are already in hand including studies of the fine radio-structure of the sun, the moons of Jupiter, and the galactic nucleus.

Vera Rich

correspondence

Travel rights

SIR,—I was very sorry to hear of the incident involving Academician Malek's passport reported by Frantisek Janouch (6 January, page 10). Ivan Malek has been a personal friend of mine for many years and I have learned to respect his wise and humane views. Personally I feel it is a tragedy that present circumstances make it very difficult for him to play the part in building up a strong, forward looking and socially concerned science in his country for which his talents so clearly qualify him.

There can be no doubt about the attitude of the World Federation of Scientific Workers toward the travel rights of scientists. I am sure Dr Janouch knows that ever since its foundation 30 years ago our Federation has worked actively for the maximum level of contacts between scientists in different countries. We fully support the UNESCO Recommendation on the Status of Scientific Researchers (1974), Article 26 of which says:

> Member states should actively promote the interplay of ideas and information among scientific researchers throughout the world, which is vital to the healthy development of science and technology; and to this end should take all measures necessary to ensure that scientific researchers are enabled, throughout their careers, to participate in international scientific and technological gatherings and to travel abroad.

Following the decision of our 11th General Assembly held in London last September, the WFSW set up a subcommittee to make these recommendations more widely known and to work actively for their practical implementation. If Dr Janouch will supply detailed, and if possible, documentary information about the incident to which he refers I am sure our subcommittee will examine it and suggest what appropriate steps, if any, the Federation should take.

E. H. S. BURHOP

World Federation of Scientific Workers,
London, UK

Toxic levels

SIR,—It is not clear what are the areas of expertise of your correspondent Thomas H. Jukes (10 February, page 491). It is clear however, that one of his areas of ignorance is chemical carcinogenesis.

How does he know that "substances that are toxic at high doses are sufficiently harmless in the amounts actually present that they offer no finite hazard to health"? This is the key to the argument about carcinogenic chemicals in the environment. They give rise to tumours in significant number when tested in small groups of experimental animals for a maximum of two years. How do we extrapolate such findings to tens of millions of people whose susceptibility is unknown but might have a wide range, both above and below that of our experimental animals, and who live for 60 or 70 years?

If we reduce the doses given to our experimental animals we reach a dose at which one or two animals in a group of 100, say, will have a tumour not seen in a group of 100 untreated controls. Although this is 1 or 2% of our population of animals (equal to 2 or 4 million of the US population), we would say that this incidence of tumours is not statistically significant, and some would claim that this dose is 'safe'. I would not. In practice, we need an incidence of tumours exceeding 5 out of 100 to be sure of statistical significance. So, because the effects of carcinogens are cumulative, we cannot establish a safe dose for any carcinogen, because we don't know how. Similarly, in our animal experiments in which we usually test single compounds we cannot take account of the probable additive or synergistic effects of several carcinogens at low doses, as we must experience them in daily life. That is why we must be wary of any carcinogen when we discover it at any level, even at the risk of appearing to be lunatic consumerists.

Perhaps Jukes should ponder the fact that there is a lot of cancer around and hundreds of thousands of people die of cancer every year, almost always of unknown cause. It is my contention that part of this cause is the carcinogenesis test which chemical manufacturers and food processors, together with Nature herself, are conducting in us, presumably with Jukes' approval.

WILLIAM LIJINSKY

Frederick Cancer Research Center, USA

Thomas Jukes replies: Lijinsky misquotes me: my statment was " . . . food contains substances that are toxic at high levels but are sufficiently harmless in the amounts actually present that they offer no finite hazard to health". With this misquotation corrected, the basis for his peroration disappears. My statement was essentially a reference to the basic principle of toxicology first enunciated by Paracelsus[1].

There are numerous examples of such substances, such as solanine (potatoes), cyanide (lima beans), goitrogens (cabbage), estrogens (soybeans, wheat germ), and selenium (marine fish). I am glad that Lijinsky included Nature in his list of providers of carcinogens. His discussion of the "safe dosage" of carcinogens alleges that "some would claim that this dose" (that is a dose that would produce tumours in "2 or 4 million of the US population") "is safe". I can think of no one who would make such a claim; certainly I would not. Various authors, including myself, have discussed the problem of reaching adequately low levels of dietary carcinogens[2-6].

It is certainly possible to postulate levels that pose a risk of less than one case per year in the US population, or that will not be effective unless we live for more than 200 years. However, it is not possible to devise a diet of natural foods that is free of "carcinogens", no matter whether they are "additive or synergistic" (as Lijinsky says), or even antagonistic (which he does not mention).

In his final sentence, Lijinsky incorrectly assumes that I approve of carcinogenic food additives. I favour bans on Reds No. 2 and No. 4, but I pointed out that many consumers apparently wanted to go on eating red maraschino cherries despite the findings on the dye. Many also seem to want cyclamates, saccharin and cigarettes. The current protest by consumers against banning saccharin is an effective but an unexpected support for the point I made. Consumerists wish to decide which carcinogens should be banned. Lijinsky often warns against bacon, but not against barbecueing of steaks, despite his own demonstration that this produces carcinogens[6], and not against tobacco, which smoked or chewed supplies nitrosamines.

A list of my publications related to cancer is being forwarded to Lijinsky.

[1] Paracelsus, Drey Bücher *Heirs of Arnold Byrckmann*, Cologne, 1564.

[2] Jones, H. B., and Grendon, A., *Food and Cosmetic Toxicology*, 13, 251 (1975).

[3] Jukes, T. H. *Preventive Medicine* 5, 438, 1976.

[4] Claus, G., Krisko, I., and Bolander, K. *Food and Cosmetic Toxicology*, 12, 737, 1975.

[5] Druckrey, H., *Potential Carcinogenic Hazards from Drugs, Evaluation of Risks*, 7, 60, Springer Verlag, Berlin 1967.

[6] Lijinsky, W. & Shubik, P. *Science* **145**, 53 1964.

news and views

Antibody production made easier

from K. I. Welsh

THE latest paper from the laboratory of César Milstein (this issue of *Nature* page 550) reporting the construction by cell fusion techniques of a line of hybrid cells secreting antibody against rat major histocompatibility antigens, promises to be an important step forward in tackling some of the many clinical and biological problems concerned with the histocompatibility system which require a reliable and standard supply of anti-histocompatibility antigen antibodies for their solution.

Tissue matching for skin, cornea, bone marrow and kidney transplants depends to a large extent on stocks of reasonable titre monospecific typing antibodies against the major histocompatibility antigens. These precious sera, obtained from immunised volunteers, multiparous women, transplant recipients or primates take months or even years of laborious work to produce, test and become acepted as standard reagents. Antibodies, over 200 in all, are tested for lysis of recipient cells and potential donor cells; identical reaction patterns indicate identical recipient and donor histocompatibility type at the HLA–A, B, C and D locus. Commercial reagents are expensive, yet the use of antisera against histocompatibility products in the analysis of the association of particular diseases with the major histocompatibility complex (MHC) as well as for routine tissue matching, is increasing daily. The ideal solution to these serological problems would be for sufficient supplies of standard typing reagents to be available worldwide.

The isolation and characterisation of MHC products, on a large scale and experiments designed to answer many basic questions related to the understanding of their evolutionary significance, present role and importance in tissue rejection and prevention of disease would also be made much easier with a readily available supply of standard monoclonal antibodies to the MHC antigens.

Milstein *et al.* constructed their histocompatibility antibody secreting cell line by fusing a mouse myeloma cell line with spleen cells of AO-strain rats previously immunised against DA strain antigens. Clones grown up from individual hybridisations were tested for their ability to produce anti-DA antibodies, and a clone was selected which was further characterised as producing antibody against an individual K/D specificity of the Ag-B4 haplotype, thereby identifying the first uniquely defined antigenic specificity in the rat MHC. The authors hope that characterisation of the products of other clones will lead to the identification of other specificities of the Ag-B4 haplotype.

This work illustrates the latest use of basic techniques developed in Milstein's laboratory for producing hybrid cell lines secreting antibody of defined specificity (see Köhler & Milstein *Nature* **256**, 495; 1975), a technique now being exploited in other laboratories.

At a recent discussion meeting at Cambridge K. Rajewsky and G. Hämmerling reported that they have hybridised tumour cells with a variety of antibody-producing cells to obtain lines of cells synthesising antibody against a wide range of idiotypes as well as bacterial and mammalian cell components.

Antibody production from tissue cultures of such cells requires culture facilities on a larger scale than many laboratories possess. Rajewsky *et al.* have however developed an *in vivo* culture system which brings production of experimental quantities of antibodies from hybrid cells easily within the capabilities of most laboratories. The hybrid cells are injected into a histocompatible animal where they proliferate, producing high levels of antibody which can be recovered from the animal's serum. This provides a method of obtaining experimental quantities of antibody from many fewer animals than needed with techniques now used.

At present, 100 rats are needed to produce 10^{5} lytic units of anti-Ag-B serum for example, but using the Milstein/Rajewsky approach only one rat would be needed, although perhaps 10 rats would be necessary to cover all the variants of specificity.

The commercial importance of such work is that it converts antibody production from a biological science with all its inherent variability and non-reproducibility into the realms of synthetic chemistry where any desired reagent is available off the shelf and can be assumed with reasonable confidence to be identical to the same reagent in any other laboratory. Monoclonal antibodies do have disadvantages; for example, a monoclonal anti-HLA-B7 reagent might only react with 10% of the HLA-B7 individuals defined by a polyclonal serum. Such disadvantages can be easily circumvented however, and there will be the advantage that it will be possible to identify the variants of histocompatibility specificities such as HLA-B7 so precisely that near perfect tissue matchings can be made, and the precise relationship of disease to the HLA-B7 complex defined.

The potential of cell fusion in immunology extends far beyond immunoglobulin production. It should eventually be possible to produce a range of helper, suppressor and factor-producing cell lines. The development of the Milstein technique now offers the British government a unique opportunity of extending this work by financing a centre for the production of such hybrid cell lines and for UK companies to take the commercial initiative. □

Neuroanatomy of visual acuity

from Peter Lennie

ONE of the aims of vision research is to find the structures that underly our visual capabilities; sometimes the relation between structure and function is striking. There is a regular correspondence between points on the retina and points on the primary visual cortex in man, but because the cortical projection of the fovea (the area of the retina responsible for the resolution of fine detail) is huge in relation to the projection of the peripheral retina, the map on the cortex is a distorted representation of the retinal surface. The large projection of the foveal region seems to be required for the resolution of fine detail, for there is a close relation between the "cortical magnification factor" (M)—the distance in mm on the visual cortex corresponding to an angle of one degree subtended at the eye—and visual acuity, expressed as the angle subtended at the eye by the smallest object that can be resolved. Cowey and Rolls (*Expl Brain Res.* **21**, 447; 1974) showed a high correlation between $1/M$ and the minimum angle of resolution (MAR) at different points in the visual field, which suggests that a constant amount of visual cortex is devoted to the minimum angle of resolution, whatever its value.

The much magnified cortical representation of the foveal region may originate in the distribution across the retina of the ganglion cells whose axons project to the lateral geniculate nucleus (LGN), which relays to the cortex. With the exception of the foveal region the density of ganglion cells decreases regularly with increasing distance from the fovea, and is found to be proportional to M^2. Thus over most of the visual field the magnification factor could be explained by assuming that, on average, each ganglion cell projects to an equal area of visual cortex. In the foveal region, where acuity is best and cortical magnification highest, the relationship between ganglion cell density and M^2 breaks down however, probably because the ganglion cells are arranged in a band around the foveal cones to which they are connected and thus are displaced from their receptive fields.

If the displacement of ganglion cell bodies causes the breakdown of the proportionality between ganglion cell density and M^2, an allowance for this displacement should extend the proportionality into the very centre of the fovea. Rolls and Cowey (*Expl Brain Res.* **10**, 298; 1970) have done this for monkeys and Drasdo (this issue of *Nature*, page 554 has now made a correction for ganglion cell displacement in man, by computing the density of ganglion cell receptive fields at different distances from the fovea. He has shown an impressive correlation between calculated receptive field density and M^2 from the fovea to points 40° in the peripheral retina, and has also formulated an equation for which he claims some generality in predicting visual performance at any point in the visual field.

These findings suggest a remarkable simplicity in the arrangement of the mechanism subserving visual acuity. However, other recent work has revealed new complexities in the structure and function of the visual system that make these simple relations rather surprising.

One of these is that retinal ganglion cells project to several areas of the brain (notably to the superior colliculus) besides the visual cortex. We cannot be clear therefore about the significance of the simple relations between visual acuity, cortical magnification and the overall density of ganglion cells until we know what fraction of cells projects to the visual cortex, and whether this fraction varies with position on the retina. The answer to this question is unclear, although recent evidence (Bunt *et al. J. comp. Neurol.* **164**, 265; 1975) suggests that in fact virtually all ganglion cells project to the cortex through the LGN and therefore that projections to other areas arise through collateral axons from the same cells.

A more intriguing problem arises from the fact that there are several different types of ganglion cell, distinguishable histologically (and in the monkey also physiologically), and so we also need to know how the different cell types contribute to the population of cells that does project to the cortex. Although it is now more than 30 years since Polyak gave a thorough anatomical description of different cell types in the primate retina, only recently has much attention been given to their possible functional roles. The impetus to much of this work was the discovery in the cat's retina of several classes of ganglion cell that are physiologically and morphologically distinctive and in some cases at least have different projections in the brain (see Levick in *Nature* **254**, 659, 1975 for a review). The primate visual system also seems to contain ganglion cell types that are physiologically distinctive (De Monasterio and Gouras *J. Physiol. Lond.* **251**, 167; 1975), although these seem to be less clearly differentiated and are not so obviously related to morphology. One is therefore led to ask what different functions they subserve and whether their projections on the primary visual cortex are topographically different.

Since the physiologically different cell types are distributed differently on the retina it seems likely that they will project differently on the primary visual cortex. This being the case it is puzzling that M, which must reflect the projections of all classes of ganglion cell, should be so simply related to MAR, which one supposes might be mediated by a single cell type. Perhaps this class is predominant, and cells of other types, even if they differ in their distribution on the retina and their projections on the cortex, occur in numbers too small to upset the simple relation between cortical magnification and minimum angle of resolution.

We might obtain some clearer idea of the functions of the putative minor classes of cell if a variety of tasks were used to examine different forms of visual acuity in relation to position in the visual field. At all events, the heterogeneity of ganglion cells argues for caution in interpreting relations between the gross structure and function of the visual pathway. □

Historical ichthyology

from a Correspondent

TAXONOMISTS are forced by the disciplines of their calling to be continually aware of the past history of their field of study. At a basic level they are obliged to refer to the earliest description or published name for an animal or plant, and possibly more than in most sciences the constant reference back to the early literature is part of the taxonomist's way of life; to a large extent the early literature is as significant as later syntheses. In this context it is not surprising that traditional taxonomists are often deeply involved with the history of their field, or with the life and work of earlier students of their subject. A recently published study of Francis Day (1829–1889) and his collections of Indian fishes by P. J. P. Whitehead and P. K. Talwar (*Bull. Br. Mus. nat. Hist. (Hist. Ser.)* **5**, 1; 1976) is an excellent example of such painstaking research.

Francis Day was born in 1829 in Sussex, and qualified in medicine at St George's Hospital in London (1851). Immediately after qualifying he joined the Indian army as Assistant Surgeon and between 1852 and 1863 worked in the medical service in India, broken only by a year's sick leave in England in 1857. Until about 1863 his career was largely medical but then he increasingly became involved in natural history studies especially those of fishes. During 1864 and 1865 he was in England, presenting his first major paper, *On the fishes of Cochin* (*Proc. zool. Soc. Lond.* **2-40**, 286; 1865), and late in 1865 publishing the *Fishes of Malabar* (Quaritch, London) a major work on Indian fishes. In 1866 he returned to India accompanying a consignment of trout eggs in an (eventually unsuccessful) attempt to establish this fish in the Neilgherry Hills. From then until 1869, although nominally still employed in the medical service of the army, Day became increasingly involved in fishery surveys and the study of fishes. In 1871 he was appointed Inspector-General of Fisheries, and commenced a series of active surveys in various parts of India. In 1874 he returned to England to begin writing *The Fishes of India* (published in four parts between 1875 and 1878, with a supplement in 1888). This book is still the only reference work on the fishes of the subcontinent as a whole, and, although many revisionary studies of groups of Asiatic fishes have been published since, reference to *The Fishes of India* is still obligatory for the student of Indian fishes.

In his later years Francis Day turned his attention to European fishes, and his *Fishes of Great Britain and Ireland* (1880–1884) and *British and Irish Salmonidae* (1887) were for many years classics in their field. They are now highly priced by the antiquarian book trade, as are his other books, which perhaps still indicates their value. Francis Day died in 1889 at Cheltenham, Gloucestershire.

These are the bare bones of Francis Day's career as surgeon and ichthyologist, and they correctly suggest a man of remarkable energy and achievement. Whitehead and Talwar provide far more detail and have produced a rounded study of the man (despite their modest statement that there is "As yet, . . . no full biography of Francis Day"), and the difficulties that he had to face with unsympathetic superiors in India, and the criticisms and obstructiveness of Albert Günther, the British Museum zoologist. Their assessment of Francis Day is that he "was one of the outstanding figures in that phase of ichthyology which called for comprehensive works on the fishes of particular regions".

Beyond the themes of biography and assessment of Day's work, Whitehead and Talwar concentrate on the distribution of Day's superlative collection of fishes. Although his collection was brought to England when he was writing *The Fishes of India*, less than half of it found its way to the British Museum. No fewer than fourteen museums received parts of his collection either by gift or sale, and his personal library and many manuscripts passed to his daughter and thence to Cheltenham Public Library. Major collections were sent to the Indian Museum, Calcutta, the Australian Museum, Sydney, the Naturhistorisches Museum, Vienna, and the British Museum (Natural History). These four collections between them hold most of the type material of species described by Day, and Whitehead and Talwar present a tabulation of all the syntypic material of Day's species in eleven museum collections. Their painstaking listing of extant specimens and the route which each lot followed to the appropriate museum, with Day's indication of their importance, is a major contribution for later taxonomists working on Indian fishes.

That so little of Day's collection (and that, in general, the less important material) was to be found in the Natural History Departments of the British Museum, later (1880) to become the British Museum (Natural History), was due to the acrimonious relationship between Day and Albert Gunther. Whitehead and Talwar deal with the beginnings of this bitter episode and its continuance until just before Day's death. The quarrel has also been touched upon recently by Günther's grandson, A. E. Gunther (*A Century of Zoology at the British Museum . . .*, Dawsons, London; 1975), but now that both sides of the story can be compared it is clear that Günther was particularly unjust in his relationship with Day. The immediate result was that the most important portion of Day's collection was sent abroad and not, as would have been expected at that time, lodged in London.

Although Whitehead and Talwar recount this episode of nineteenth century zoological in-fighting in detail, and one must admit it makes fascinating reading, their study of the history of Day's collection is a fundamentally important contribution to furthering the study of the taxonomy of Indian fishes. □

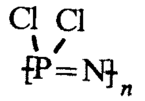
Polyphosphazenes: tailored polymers

from Paul Calvert

The polyphosphazenes are a versatile group of polymers based on an alternating phosphorus and nitrogen chain, which are potentially useful as low temperature, solvent resistant elastomers and may also have a number of applications in medicine. Recently published calculations of conformational energies shed light on the origin of their rubber-like behaviour and nicely illustrate both the gap between calculable and observable quantities and the benefits which could be gained by closing the gap.

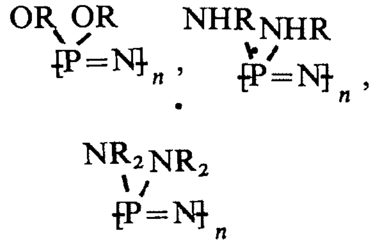
Inorganic polymers have been a subject of interest for many years, partly in the hope of finding materials which would be resistant to temperatures above 350 °C where most organic polymers start to oxidise and partly, one suspects, to allow inorganic chemists to make themselves useful. The effort has not been a great success, to a large extent because many of the polymers produced are thermally stable but very vulnerable to hydrolysis. The only important group of commercial inorganic polymers is the silicone rubbers which are used as low temperature, chemical and solvent resistant elastomers (glass transition temperature 150 K). Their main drawback is that they are mechanically weak.

The polyphosphazenes have the greatest promise of the remaining inorganic polymers. The simplest example is poly(dichlorophosphazene) produced



$$\left[\overset{\mathrm{Cl}\ \mathrm{Cl}}{\mathrm{P}}{=}\mathrm{N}\right]_n$$

by polymerisation of the trimer hexachlorocyclophosphazene which is the product of reaction between ammonium chloride and phosphorus pentachloride. This polymer readily hydrolyses starting at the chlorine–phosphorus bond but the trick to producing stable polymers found by Allcock and coworkers in 1965 is to replace the chlorine with organic groups linked to the chain by either an oxygen or nitrogen atom. This is done by reacting the chlorinated polymer directly with the appropriate amine or sodium alkoxide.



$$[\mathrm{P(OR)_2{=}N}]_n, \quad [\mathrm{P(NHR)_2{=}N}]_n, \quad [\mathrm{P(NR_2)_2{=}N}]_n$$

Since then Allcock's group at Pennsylvania State University have published about 30 papers describing the synthesis and properties of a large number of these polymers (see *Science* **193**, 1214, 1976; and *Chemtech* 553, September 1975). The interest of these polymers lies in this versatility, an indefinite number of side groups can easily be added to produce a wide range of properties without having to perform a new polymerisation each time. The low temperature flexibility of some of these materials can also be applied.

Low temperature elasticity is necessary not only to make rubber grommets for use in cold climates but also in the pumping and handling of cold liquids. A necessary condition for this is believed to be chain flexibility, that is, unhindered rotation about the chain backbone bonds. In the polyphosphazenes and silicone rubbers this arises by the alteration of an unsubstituted chain atom (the nitrogen) with one carrying only small substitutes (the phosphorus) so that internal rotation can occur without the side groups colliding. Allcock, Allen and Meister (*Macromolecules* **9**, 950, 956; 1976) have made a start toward quantifying this by calculating the potential energy as a function of conformation for a series of halo- and organo-substituted polyphosphazenes. These calculations assume a Lennard-Jones interatomic potential and then evaluate the potential energy due to neighbouring groups on the chain as a function of the bond angles. However the chain does have a conjugated double bond structure which might also hinder rotation, although Allcock asserts that it does not on the basis that the orbitals will be symmetric about the bond axis. The exact nature of the P–N double bond is still a matter for debate and they may have to be taken into account (Allcock *Phosphorus–Nitrogen Compounds* Academic, London–New York, 1972). The potential energy barrier to bond rotation calculated in this way is directly related to the chain flexibility and can be compared with the polymer glass transition temperature which varies from 180 K for the difluoro polymer to 265 K for poly(diphenoxyphosphazene). The calculated barrier heights correlate well with the observed glass transition temperatures for the halo- and organo-substituted polymers separately but poorly when the two groups are compared, the organo compounds having larger barriers to rotation for similar glass transition temperatures. Clearly some other factor must be taken into account.

This question should really be taken further as there has been relatively little advance since the theory of Gibbs and DiMarzio in 1962 stressed the importance of chain flexibility for the glass transition. The polyphosphazenes provide a good system on which to relate properly the calculated conformational energies to the glass transition temperatures since the ease with which substituents can be changed on the same backbone should allow the isolation of factors such as polarity, side group flexibility and copolymer composition.

The proposed uses for the polyphosphazenes also reflect the effect of side groups on the polymer properties. Small side groups give a low glass transition temperature and resistance to swelling by hydrocarbons, desirable for gaskets and O-rings. Crystallisation is prevented by the use of mixed substituents so that the polymer is rubbery With some subtlety it should be possible to produce a rubber which crystallises only on stretching. This would give good strength properties comparable to natural rubber rather than to silicone rubber which cannot crystallise and is weak. By adding fluorinated substituents polymers can be produced with a low surface energy which could be used for water repellent coatings and for surgical implants

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Ultraviolet windows in sunglasses

from John Walker

CAN sunglasses damage the eyes? Two American scientists have discovered that many sunglasses on sale in the United States are not effective in filtering out ultraviolet radiation; they may actually increase the possibility of eye damage in bright sunlight.

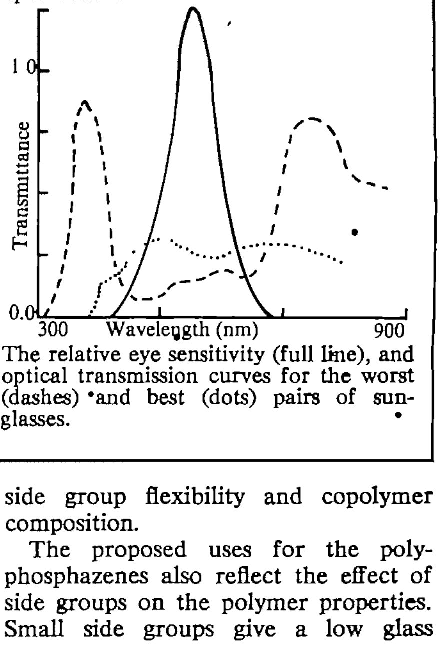
Visible light has wavelengths between 400 nm (blue/violet) and 700 nm (Fig. 1). Other wavelengths, which are at best unnecessary for vision and at worst a danger to the eyes, ought to be removed by sunglasses before reaching the eyes. Ultraviolet (300–400 nm) in particular is suspected of causing cataracts.

Anderson and Gebel (*Appl. Optics* **16**, 515; 1977) tested sunglasses they had randomly purchased in local shops and drugstores (in Ohio, USA). Many of them were optically unsatisfactory and " . . . seem to be designed more to satisfy the fashion whims of the wearer than to shield the eyes from unnecessary radiation". One-third of their selection transmitted more light in the ultraviolet than in the visible—in the worst case 70% transmission at 360 nm compared with 20% at 550 nm (Fig. 1). This may be dangerous for two reasons.

First, as the amount of visible light reaching the eye is cut down, much stronger outside light levels can be tolerated without discomfort, and so the ultraviolet exposure may be greater than if no sunglasses were worn. Alternatively, the reduced visible light intensity causes the pupil to dilate allowing more light to enter the eye. Anderson and Gebel calculate that the worst sunglasses actually double the amount of ultraviolet absorbed by the eye (Fig. 1); thus there is a greater risk of eye damage than if no sunglasses were worn.

Some sunglasses were quite satisfactory, however. Figure 1 shows the transmission spectrum of one of the better pairs—though even there it would be better if the transmission cut off at 450 nm instead of 390 nm. And unfortunately there appeared to be no correlation between ultraviolet performance, price, lens colour or manufacturer; there is no way of picking a good pair other than by measuring its transmission spectrum.

The authors conclude that their preliminary study should be extended, and should include prescription sunglasses (the only pair they tested performed very well). Furthermore "... it would be in the consumers' interest for manufacturers to indicate in some manner the transmission characteristics of their lenses over the solar spectrum".



The relative eye sensitivity (full line), and optical transmission curves for the worst (dashes) and best (dots) pairs of sunglasses.

where a low surface energy is believed to reduce the tendency of implants to cause blood clotting. The amino-substituted polymers can be made water soluble and are susceptible to hydrolysis to varying extents. Uses as resorbable sutures and slow release drug carriers are being investigated.

There are a number of groups of polymers where a wide range of properties can be produced by altering the side groups. The proteins and polysaccharides are two classes which have proved their value over the years. Vinyl polymers which range from polyethylene to polyacrylamide could also be seen as such a class Polyphosphazenes are unlikely ever to be used on a large scale but they do seem to have promise in terms of tailoring polymers for specific, small scale uses. Here the ability to add substituents without having to perform a new polymerisation is a real advantage. This approach of designing polymers for specific purposes would be real molecular engineering, something that is more often talked about than practised. The snag is that our current methods of predicting properties such as solubilities and transition temperatures are so crude as to shame most other branches of engineering. ☐

Parental care

from John Krebs

One characteristic of higher vertebrates is that they usually look after their eggs or young, a trait which is rather rare in fish, amphibians, and reptiles. The advantages of parental care in terms of survival of young may be obvious, but it is less clear why one sex rather than the other should provide the care. In birds and mammals the trend is for females to do more than males for their young, while in fish it is often the other way round.

The theory of natural selection leads one to expect that each parent should, at any time during its reproductive life, adopt the course of action which maximises the number of young produced in the remainder of its career. (It should not, of course, make the Concorde Fallacy of directing its future action on the basis of past investment (Dawkins & Carlisle *Nature* **262**, 131; 1976)). For example, if the survival of young is greatly enhanced by care from one parent, but the second parent makes rather little difference, it would pay each parent to try and leave the young in the care of its partner, to go off and mate again. On the other hand if the survival of young depends (as in most birds) on care from two parents, it will pay neither to desert.

An analysis of the theory of parental care and desertion has recently been published by J. Maynard Smith (*Anim. Behav.* **25**, 1; 1977). He considers two situations in which breeding is synchronised and seasonal: in one the factor-limiting production of young is parental care, whereas in the other, the number of eggs laid by the female is also important. In the first model, it would not pay the female to produce extra eggs since they would not survive without additional care. The first model applies to most birds. Birds are usually monogamous and both parents care for the young, but if one parent can look after the young (as in species with precocial young such as gallinaceous birds) and there is a reasonable chance of a second mating, it is usually the male which deserts. Although it would equally pay the female to desert, she is subject to a time constraint: she has to wait after copulation until she has laid the eggs, and this may mean that she is too late to have a chance of finding a second mate when there is synchronised seasonal breeding. The female can however encourage male fidelity by delaying tactics. If she refuses to copulate until the male has little chance of finding a second unmated female, she may force him into faithful monogamy.

In most fish, and a few birds, parental care takes the form of guarding and not feeding, so that the number of young reared by a female will depend not solely on the amount of parental care, but also on the initial production of eggs. Here the female has, so to speak, a choice of directing her reserves into parental care and laying fewer eggs, or channelling all her reserves into eggs and deserting each clutch. As long as one parent can rear the young, it will pay the female to desert, which she often does. As pointed out by Dawkins and Carlisle (*op. cit.*), an additional factor favouring male parental care in fish is external fertilisation. With external fertilisation the male has to deposit his sperm after the eggs have been laid; as a result the female has the chance to desert first, just as in species with internal fertilisation the male has first chance.

Mammals are rarely monogamous; male desertion is common because of the physiological commitment of the female to parental care, at least in the early stages. There are, however, some monogamous mammals, such as the gibbons (*Hylobates* spp.), and it is perhaps somewhat surprising that these species have not evolved more male parental care, for example in the form of male lactation. ☐

Neural systems

by Miranda Robertson

The Dahlem Conference on the Formation and Function of Neural Systems was held in Berlin on 7–11 March, 1977. The proceedings will be published and can be obtained from Dahlem Konferenzen, Delbruckstrasse 4c, D-1000 Berlin 33.

Although nominally the meeting was addressed to the function as well as the formation of neural systems, it was effectively dominated by the question of formation.

The growing contribution of genetics to the issue was divided by Peter Lawrence (MRC Cambridge) into two categories: the identification of genes which control development directly, and the identification of crucial cellular interactions through neurological mutants in which they have gone astray.

Two recent advances discussed at the meeting fall into the first category. One is the addition of isogenic locusts to the menagerie of mutants, mosaics and isogenic invertebrates on which much neurodevelopmental insight depends. A series of isogenic strains has been used by C. Goodman (in experiments described by D. R Bentley, University of California) to distinguish genetically controlled variation from epigenetic 'noise' in the determination of an identified neurone. While the position of the cell body varied only between strains (implying genetic control), the terminal branching of the axon varied between three positions, at different axonal lengths, within strains (implying epigenetic control).

Deducing the nature of the genetic control from mutants is, according to Lawrence, enormously complicated by the fact that most developmental mutations are bound to be lethal, which means that survivors are probably 'leaky' mutants and the investigator is thus faced with deducing wild-type function from the effects of its reduction rather than its total absence. The problem was well illustrated by the effects of homoeotic mutations on the nervous system of *Drosophila* (J. Palka, Washington University). Homoeotic mutations transform one entire structure into another—for example, wing into haltere; but the innervation of the mutant structures seems to bear no consistent relationship to the target tissue, since it can correspond either to that of either the wild-type or the mutant appendage, or to a mixture of the two, sometimes in conjunction with an abnormal pattern unlike either.

Genetic mosaics, which can be used to circumvent the lethal consequences of developmental mutations, can also distinguish direct from indirect effects of such mutations. Myerowitz and Kankel (as reported by F. D. Ready (Stanford University Medical School)) have found, for example, that a small group of mutant retinula cells in the eye of *Drosophila* mosaics causes a 'cascade' of abnormal projections in the first and second optic ganglia, although all the other cells involved are wild type.

A similar 'cascade' seems to characterise the visual system of the Siamese cat, in which a discrete subset of optic nerve fibres which projects ipsilaterally in normal animals instead crosses at the chiasm. This gives rise to abnormal connections in the lateral geniculate and at the cortex, where the callosal connections have now been shown to be abnormal as well (C. Shatz *J. comp Neurol.* **171**, 229; 1976). This abnormality is common to albinos of all species so far tested and is known to result from the absence of pigment rather than from the action of the albino gene itself, or the tysosinase deficiency which is its direct effect. (The incrimination of the pigment is due to numberless mink-breeders, who have produced animals synthesising pigment in varying quantities through mutations in no less than five different genes; and to R. W. Guillery, who has established that the degree of optic fibre misrouting in minks corresponds to the pigment deficiency and not to any particular gene.)

Guillery (University of Wisconsin), following up this clue to the cause of the mis-specification of the retinal fibres, has found that (in hamsters) a small group of pigment epithelium cells is born and dies at the base of the optic stalk at a time when no other pigment tissue has yet appeared, but the optic fibres are just beginning to grow out from the eye. It seems possible that those pigment cells induce the specification of the ganglion cell axons.

Another example of induction of neural function by non-neural cells is that of the induction of biochemical differentiation in superior cervical ganglion cells *in vitro*. Rat sympathetic neurones cultured on their own are adrenergic, but when grown in medium 'conditioned' by various types of non-neural cell they synthesise acetylcholine and form nicotinic cholinergic synapses (Furshpan *et al Proc. natn. Acad Sci. U.S.A.* **73**, 4225; 1976). Further experiments with these cells (P. Patterson, Harvard Medical School) have shown that all tissues seem to be able to induce cholinergic function, though to widely varying degrees which do not seem to be related to whether they normally receive cholinergic or adrenergic innervation, or any innervation at all There is evidence in the spinal cord *in vivo* that transmitter induction is determined by position (Le Douarin *et al. Devl Biol.* **41**, 162; 1974).

The question of how far neural development is dictated by other tissues arises in a more specific form in the context of synaptogenesis, which has been investigated principally at the neuromuscular junction. Do neurones, for example, recognise their target tissue by its neurotransmitter receptors? Experiments showing synaptogenesis in the face of receptor blockade imply that if they do, it is not by virtue of their transmitter binding sites (Cohen *Brain Res.* **41**, 457, 1972; Anderson *et al. Neurosci. Abstr.* **2**, 707; 1976). The possibility, fleetingly raised, that receptor clusters (hotspots) might attract innervating fibres has also been ruled out by Anderson *et al.*, who showed by fluorescence labelling of receptors during synaptogenesis that innervating fibres did not make synapses at existing hotspots but induced receptor clustering very rapidly on contact.

It is now clear that an important contribution to the pattern of synapse formation is made by the withdrawal of most of the contacts initially made by the innervating neurone. The evidence for this selective elimination comes both from physiological and anatomical investigations on the neuromuscular junction (Brown *et al. J. Physiol., Lond.* **261**, 387; 1976) and from anatomical studies on the cat visual system (Rakic *Nature*, **261**, 467; 1976, Hubel *et al. Phil Trans R. Soc.* in the press). Its mechanism is not known, but seems to reside largely in the nerve. Brown *et al.* have ruled out competition between lenses by the demonstration of withdrawal of synapses from rat soleus after 75% of the innervating axons have been cut, even when that leaves many fibres denervated. They do not however rule out some influence of the muscle itself in the selection of which fibres are to be retracted.

Investigation of this phenomenon in nerve–nerve synapses has been made possible for the first time by the discovery of J. Lichtman, reported by D. Purves (Washington University), that four out of five terminals made by guinea pig submandibular ganglion cells are withdrawn within four weeks after birth.

The broader implications of selective synaptic elimination are suggested by the proposal (D. Hubel, Harvard Medical School) that the period over which it occurs may correspond to the 'critical' period within which the development of the central nervous system can be influenced by its input. But the demonstration that the effects of monocular deprivation can be reversed during the critical period implies that fibres must be able to sprout as well as to withdraw.

So far, anatomical evidence of plasticity has been available only for the distribution of ocular dominance in the innervation of the visual cortex and anatomical data on the distribution of orientation selectivity (the nature of whose plasticity has been fiercely disputed) have been beyond neurology's technical means. Now however the anatomy of the orientation columns has become accessible through the discovery that ^{14}C-deoxyglucose can be used for the selective staining of active neurones (Kennedy *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4230; 1976). Hubel has applied this technique to the autoradiographic mapping of the vertical orientation columns of the monkey visual cortex, confirming the indications from electrophysiological mapping that there is no regular anatomical relationship between the orientation columns and the ocular dominance columns. Whether this technique can be brought to bear on the issue of plasticity in orientation selectivity remains to be seen.

In the meantime, however, invertebrate neurophysiologists are preparing to join the fray. R. Murphey (State University of New York) reported that unilateral immobilisation of the mechanoreceptive hairs on the cerci of developing crickets raises the sound threshold of the medial giant interneurone which receives its input from the cercal mechanoreceptors on the treated side of the animal. Matsumoto and Murphey (*J. Physiol., Lond.* in the press) suggest on the basis of the directional tuning curve of the giant neurone that an increase in the activity of the crossed inhibitory input from the contralateral cercal hairs may at least partly account for their result.

While this has interesting echoes of the suggestion (Duffy *et al. Nature* **260**, 256; 1976) that in monocular deprivation, a tonic inhibitory input to the visual cortex from the non-deprived eye may explain the unresponsiveness of deprived cortical cells, there is much too much uncertainty about the precise mechanism of either the invertebrate or the vertebrate results to justify firm conclusions, let alone direct comparison, at this stage. □

articles

Thermal evaporation of gas within galaxies by a hot intergalactic medium

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High-temperature ($T \sim 10^8$ K) intergalactic gas interacts with cooler embedded gas within galaxies and extragalactic clouds principally by thermal evaporation. From Cowie and McKee's model for evaporation of spherical interstellar clouds, in pressure balance with a hot ambient medium, we produce solutions for oblate and prolate spheroidal symmetry. The results are applied to evaporation of gases within spiral and elliptical galaxies, and can be extended to evaporation of filamentary clouds in the interstellar medium.

THE recent discovery of Fe XXV emission lines in the spectrum of X-ray emission from the Perseus cluster[1], together with measurements of the cosmic microwave background[2], thermal bremsstrahlung spectrum fitting[3], and tailed radio galaxy measurements[4], provides convincing evidence for the existence of a hot ($T \sim 10^8$ K) intergalactic gas within the potential well of rich clusters of galaxies. It is also likely that a substantial amount of gas, residual from the galaxy formation process, lies outside the rich clusters; such gas may provide the closure density required to bind the universe gravitationally. Field[5] has summarised the observational constraints on such gas and shown that for a Hubble constant of 50 km s^{-1} Mpc^{-1}, (a critical hydrogen number density of 2.6×10^{-6} cm^{-3}), observations require only that the gas be highly ionised at $z = 2$ and at temperatures of less than 4×10^8 K at the present epoch. (For higher values of the Hubble constant, constraints imposed by the isotropic X-ray background are more severe, since the intensity is proportional to $H_0{}^3$). The most attractive evidence for the presence of such an intergalactic medium is the feature observed at 30 keV in the isotropic X-ray spectrum which may be fitted well by thermal bremsstrahlung from a gas with closure density and a present temperature of 3×10^8 K (refs 5, 6).

Several studies have been made of the thermal evaporation of gas within galaxies[7–9], and of the ionisation by the metagalactic ultraviolet radiation which dominates at lower temperatures[10]. De Young[7] and Bergeron and Gunn[8], analysed the evaporation as direct particle heating by penetrating particles (ions and electrons) from the intergalactic medium, neglecting the dynamics of the evaporation process. An analytical model for evaporation has been developed by Cowie and McKee[11], who derived time-independent evaporative mass loss rates for a spherical cloud embedded in an infinite hot gas with asymptotic density n_f and temperature T_f by finding simultaneous eigenvalues of the mass and energy conservation equations

$$\nabla.(\rho \mathbf{w}) = 0 \qquad (1A)$$

$$\nabla.\rho\mathbf{w}\,(\tfrac{1}{2}\mathbf{w}^2 + 5/2\,c^2) + \nabla.\mathbf{q} = 0 \qquad (1B)$$

where ρ is the mass density, w the velocity of outflow, c the isothermal sound speed and q the electron heat flux.

In the case in which the heat flux can be written as

$$\mathbf{q} = -\kappa\nabla T \qquad (2A)$$

where κ is the classical thermal conductivity

$$\kappa \equiv k_0 T^{5/2} = (1.84\times10^{-5}\,T^{5/2})/(\ln\Lambda \text{ erg s}^{-1}\text{ deg}^{-1}\text{ cm}^{-1}) \qquad (2B)$$

($\ln\Lambda$ is the Coulomb logarithm, ref. 10) the pressure is approximately constant throughout the interface and the flow subsonic; since the condition $M^2 \ll 1$ is satisfied, equations (1A and 1B) are approximately separable in a number of coordinate systems. (The equations cease to be separable when the term $(\mathbf{w}.\nabla)\,\mathbf{w}$ in the momentum conservation equation becomes important.)

Here we shall describe the solutions for both oblate and prolate spheroidal symmetry. The analysis closely follows that in Cowie and McKee[11]; in particular, oblate (prolate) spheroidal surfaces of constant u replace surfaces of radius r (the notation is that of Arfken)[13]. Thus the boundary conditions become $T = 0$ at the surface of the cloud ($u = u_0$) and $T \rightarrow T_f$ as $u \rightarrow \infty$. In terms of u_0 the mass loss rates from the clouds are

$$\text{Oblate: } \dot{M} = 8\pi a\mu k_0\, T_f{}^{5/2}/25\, k_B(\pi/4 - \theta_0) \qquad (3A)$$

$$\text{Prolate: } \dot{M} = 16\pi a\mu k_0\, T_f{}^{5/2}/25\, k_B\, |\gamma_0| \qquad (3B)$$

Here μ is the mean mass per particle, k_B the Boltzmann Constant, while

$$\theta_0 = \tan^{-1}(\tanh\tfrac{1}{2}u_0) \qquad (4A)$$

$$\gamma_0 = \ln(\tanh\tfrac{1}{2}u_0), \qquad (4B)$$

where a is related to the semi-major (l) and semi-minor (b) axes of the ellipsoid of constant u by

$$l = a\cosh u \qquad (5A)$$

$$b = a\sinh u \qquad (5B)$$

In the limit as the surface $u_0 \rightarrow 0$ (oblate spheroid → disk, prolate spheroid → needle), the result for the oblate case is a factor of $2/\pi$ times the mass loss rate from the surface of a sphere of radius a; however, in the prolate case, the mass loss rate tends to zero as the surface area of the cloud vanishes. Both mass loss rates drive clouds towards sphericity on timescales comparable with the evaporation time.

We have also evaluated the temperature distributions as

Oblate
$$(T/T_f)^{5/2} = \frac{\theta_o - \theta}{\theta_o - \pi/4} \quad (6a)$$

Prolate
$$(T/T_f)^{5/2} = \frac{\gamma_o - \gamma}{\gamma_o} \quad (6b)$$

where θ and γ refer to the surface of constant u at which the temperature is evaluated. These may be used to evaluate the column density of a given ion in the evaporative interface (see Section III of McKee and Cowie[14] for details). For example, along the axis $\nu = \pi/2$ of the oblate spheroid (the axis of rotational symmetry), we obtain for the column densities of OVI, assuming cosmic abundances[15] and steady-state ionisation equilibrium[16]

$$N_{\mathrm{OVI}} = 2.04 \times 10^{-4}\, a n_f (3 \times 10^5\ \mathrm{K}/T_f)^{3/2} \quad (7)$$

Emission measures may also be calculated in terms of the external density and temperature (n_f, T_f) and the dimension a.

The mass loss rates calculated above may overestimate the mass loss if the heat flux given by the classical formula of equation (1) exceeds the maximum heat flux which may be carried by the electrons. This was estimated by Cowie and McKee to be $5\varphi_s \rho c^3$, where φ_s is a factor of order unity. In the spherical case the mass loss rate may be calculated exactly even in this limit (compare Cowie and McKee) [11]; however, in the geometries considered, saturation of the conductivity is a symmetry breaking effect which precludes an analytic solution, while, as we have discussed above, the hydrodynamic equations are no longer separable.

Thus we note that, in the oblate spheroidal geometry, saturation of the conductivity occurs initially along the $\nu = 0$ axis, where temperature gradients are a maximum. In terms of the parameter

$$\sigma_o = \frac{2\kappa_f T_f}{25\varphi_s \rho_f c_f^3 a} \simeq \left(\frac{T_f}{1.54 \times 10^7}\right)^2 a_{\mathrm{pc}}^{-1} n_f^{-1} \quad (8)$$

which measures the ratio of the electron mean free path to the characteristic dimension of the system, this occurs when

$$\sigma_o \sim 5.7\, u_o^{4/5},\ u_o \ll 1 \quad (9)$$

or substantially earlier than would be the case for a sphere of radius a where saturation occurs at $\sigma_o \sim 1$. Initially the effect is confirmed to a very narrow range of ν and has little effect on the mass loss rates. As $\sigma_o \to 2.5$ the heat flux saturates throughout the interface; beyond this range we would expect the mass loss rates to approximate those found by Cowie and McKee[11] for spherical clouds of radius a, because the energy flow is governed by the outer regions of the saturated zone which is approximately spherically symmetric.

For clouds of a given column density, the effects of radiation on the evaporation depend only on the external temperature[14]; numerical integration shows that the results of McKee and Cowie for the radius of spherical clouds at which radiation dominates the conductive heat flux and clouds condense are approximately applicable to the oblate spheroidal geometry, if the dimension a is substituted for the spherical cloud radius. For temperatures $>10^6$ K, the condition for radiation to be unimportant is that the factor σ_o exceeds 0.027. We have also assumed in the above discussion that the effects of magnetic field structure do not substantially inhibit the thermal evaporation. Cowie and McKee suggest that this approximation is reasonable if the magnetic fields of the cloud and intercloud are well connected, a condition that will be self consistently satisfied if evaporation is important. For the present purposes it may be considered as an extreme assumption, providing an upper bound to the mass loss rate by evaporation.

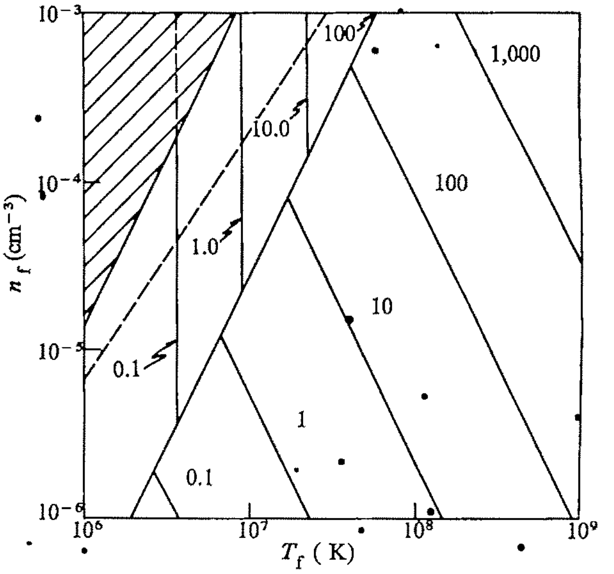

Fig. 1 Evaporative mass loss rates (in $M_\odot$ yr^{-1}) from a spiral galaxy of radius 15 kpc as a function of the local intergalactic temperature and density. The diagram is divided into three zones; in the shaded region radiation from the interface is important and galactic material condenses on to the galaxy, in the central region classical mass loss rates are applicable, while to the right of the solid line flux limiting effects are important (see text). We also show (— — — — —) the value of the external parameters for which absorption lines in the interface may be detectable ($N_{\mathrm{OVI}} = 10^{13}$ cm^{-2}).

We note finally that the evaporation rates given above for clouds in pressure equilibrium with the ambient gas should also be applicable to the evaporation of gas within galaxies, because, if the external temperature of the gas is large by comparison with the velocity dispersion of the galaxy, temperatures in the interface rise too rapidly for the gravitational field of the galaxy to be important.

In Fig. 1 we show the mass loss rate from a spiral galaxy of disk radius 15 kpc and thickness 200 pc for a wide range of intergalactic temperatures and densities. For $\sigma_o > 1$ (shown by the dashed line), we have used the spherically symmetrical results of Cowie and McKee; the discontinuity across this surface reflects the uncertainty in the mass loss rates. To within the factor of $2/\pi$ introduced by the geometry, the results may also be applied to galaxies of arbitrary ellipticity.

The mass loss rates are a sensitive function of the local temperature, but somewhat less sensitive to the local density. The existence of cool gas within galaxies therefore provides an upper bound to the local temperature of the intergalactic gas which is a weak function of the intergalactic density. The most sensitive probe is provided by the observations of column densities of 2–3×10^{19} cm^{-2} (0.18–0.27$M_\odot$pc^{-2}), of neutral hydrogen by Roberts and Whitehurst[22] at 30 kpc from the centre of M31, where the surface density of material inferred from the rotation curve is 30$M_\odot$pc^{-2}. The column density of ionised shielding slabs is unlikely to greatly exceed this value[24]. In the absence of replenishment by the stellar population, the evaporation time for the neutral hydrogen alone equals the Hubble time at a local temperature of 3×10^6 K and is independent of the density for $n \geq 2 \times 10^{-6}$ cm^{-3}. This value is comparable to that obtained by Cowie and McKee[9] from consideration of extragalactic clouds. The present results, however, are significantly more uncertain, as the possibility of substantial mass loss from a stellar population cannot be excluded. De Young[7] estimates that if the 30$M_\odot$pc^{-2} is composed of 0.1$M_\odot$ M type dwarves losing mass at a rate of $10^{-12} M_\odot$ yr^{-1} comparable with the solar value, an upper bound to the mass loss rate is $3 \times 10^{-10} M_\odot$ yr^{-1} pc^{-2}. This would result in the weaker condition $T \leq 10^7$ K for $n = 3 \times 10^{-6}$ cm^{-3}.

It must be stressed, however, that these values refer only to the temperature of intergalactic gas within local groups. We emphasise that the average temperature of the intergalactic gas may be substantially higher, though temperatures as high as 3×10^8 K, required to explain the isotropic X-ray background, seem to be ruled out by the upper bounds on cooler material within groups, provided by soft X-ray measurements (compare Cowie and McKee[9]). The values of the mass loss rates from the galaxies are sufficiently large that evaporative stripping could clear material from galaxies in regions where intergalactic gas temperatures are high, and could account for the absence of gas within field SO galaxies.

We note that for local intergalactic densities of order 3×10^{-6} cm^{-3} neither emission nor absorption in the interface will be detectable, irrespective of the ambient temperature. If lower temperature, higher density gas does exist within the local cluster, the interface might be detectable in absorption (Fig. 1).

We may apply similar arguments to galaxies which are members of rich clusters. The parameters of the intracluster medium in the central regions of the cluster, based on the thermal bremsstrahlung interpretation of the X-ray emission from the Coma cluster[18] are $n \sim 3\times10^{-3}$ cm^{-3} ($\Omega \sim 1{,}000$), and $T = 10^8$ K. The ratio of the mass loss rate by thermal evaporation to the stellar mass injection is maximised if the cool gas is confined to the nucleus of the galaxy. The evaporation from the nucleus however, is $1.4\times10^{-4}\ R_p{}^{1.625}\ M_\odot\ \mathrm{yr}^{-1}$ (Fig. 1), where R_p is the core radius of the galaxy in pc; for $R_p = 100$ the mass loss rate is $0.25 M_\odot\ \mathrm{yr}^{-1}$. Thus even in the most favourable case for retaining gas, the evaporation rate exceeds the probable mass injection rate by stars inside the nucleus for all but the most massive galaxies.

We suggest that this effect rather than ram pressure stripping is responsible for the absence of the OII λ3727 line in elliptical galaxies within rich clusters, because, as Gisler[23] has pointed out, ram pressure stripping is ineffective in removing pre-existing gas from the nuclei of galaxies. In a sample of 100 galaxies within the Coma cluster by Sargent and Gunn (quoted in ref. 19) this line was detected only in the giant central galaxies NGC4889 and NGC4874, which is consistent with the present results.

Finally, we may note that comparison of the critical radius for radiative stabilisation with characteristic scales of the galaxies (Fig. 1) indicates that radiation is never important, even for the giant central galaxies. The results indicate that the cD galaxies are not now growing by conductive condensation of hot intracluster gas, as has been suggested[19], although radiative cooling of the central intracluster gas on scale lengths large compared with the radii of these galaxies may ultimately result in gravitational accretion of material on to these galaxies[21,25].

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Geochemical evolution during fractional crystallisation of a periodically refilled magma chamber

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A realistic model of volcanic plumbing predicts that most of the established major, trace and isotopic chemical features of the common basalts could have been imposed during magma evolution in high-level magma chambers, for which process there is extensive independent field and phase equilibria evidence. These effects must be quantified before chemical features of lavas are ascribed to the nature of the underlying upper mantle.

THE changes in the chemistry of basaltic liquids which occur during a variety of partial melting processes have been explored in mathematical models[1,2]. Extensive variations in the concentrations of those incompatible trace elements which are strongly concentrated into the liquid are observed to occur with little accompanying change of the concentrations of the major elements (notably Fe, Mg, Na, Ca), particularly where the percentage of partial melt is small. Similar results can be produced by interaction of basalt magma with a peridotite wall rock in a process akin to zone refining[3]. General geological constraints suggest that both partial melting and zone refining processes have been restricted to relatively high pressure environments in the Earth.

The argument outlined here is developed in the particular context of basaltic magmas but the model presented is equally applicable to any magma, because it is a mathematical formulation of the effects which will follow from a particular style of plumbing in a volcano.

Basaltic liquids which are isolated from their upper mantle source regions may undergo crystallisation either while full equilibrium is maintained between the residual liquid and the crystals which have formed, or in such a way that all crystals formed are immediately removed from contact with the liquid. The changes in the chemistry of the liquids during these processes differ strikingly from those produced in partial melting processes. Large variations in the concentrations of the incompatible trace elements can occur, but only at the expense

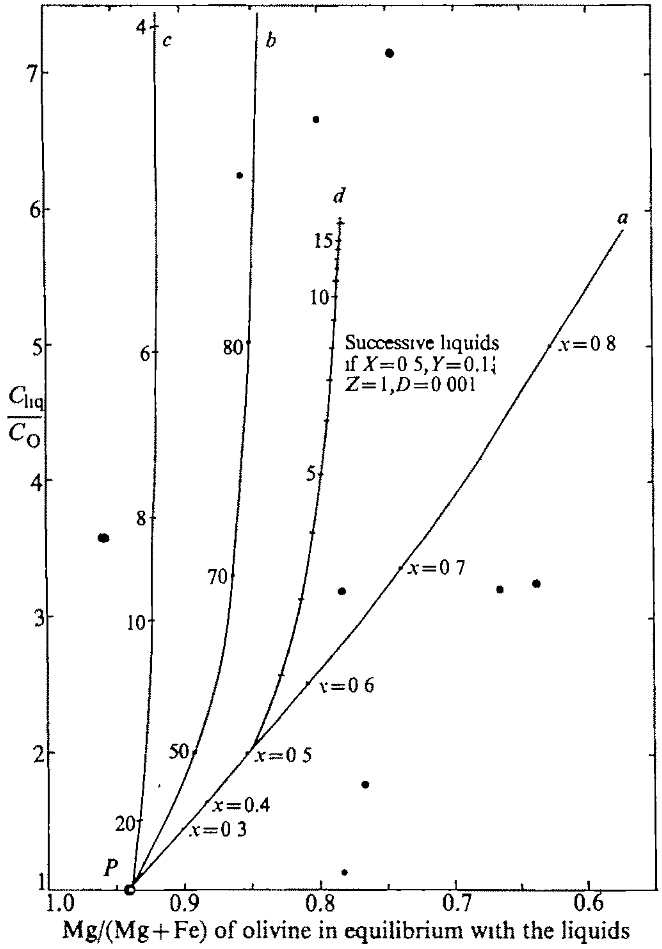


Fig. 1 How the relative concentration of an incompatible trace element: ($D = \frac{\text{conc. element in crystals}}{\text{conc. element in liquid}} = 0.001$) will vary in the liquid fraction during a variety of processes which may lead to the development of a liquid P, and a further group of processes which may act on a liquid such as P. The change of concentration of the incompatible trace element relative to its concentration in liquid P is plotted as a function of the ratio of two major, relatively compatible elements, exemplified by Fe, Mg for which it has been assumed that $D = 0.7, 2.1$, respectively. The scale is, however, translated into one showing the composition of the olivine which would coexist with these liquids, assuming

$$K_D = 0.33 = \left(\frac{\text{Fe}}{\text{Mg}}\right)_{\text{ol}} \left(\frac{\text{Mg}}{\text{Fe}}\right)_{\text{lq}}$$

It has been assumed that liquid P is in equilibrium with olivine of composition Fo_{94}. Further discussion is in the text.

of producing large volumes of crystal cumulates, and extensive increases in the ratios of major elements such as Fe/Mg, Na/Ca. General geological constraints suggest that such processes occur extensively at low pressures, and are probably important at high pressures also.

The differences between the chemistry of the liquid products of the partial melting, equilibrium and fractional crystallisation processes are illustrated in Fig. 1, curves *a*, *b*, *c*. Curve *a* shows the evolution of the residual liquid composition during closed system perfect fractional crystallisation (with percentage crystallisation marked). Curve *b* shows the evolution of the intercrystalline liquid during perfect equilibrium crystallisation (with percentage crystallisation marked). Curve *c* shows the variation during an equilibrium partial melting process which would yield P as a 30% melt of the source region (with intermediate compositions and the percentages of melt then formed indicated).

None of the processes so far considered are effective in causing a variation in the ratio of two elements which are both strongly concentrated into the liquid relative to the crystals.

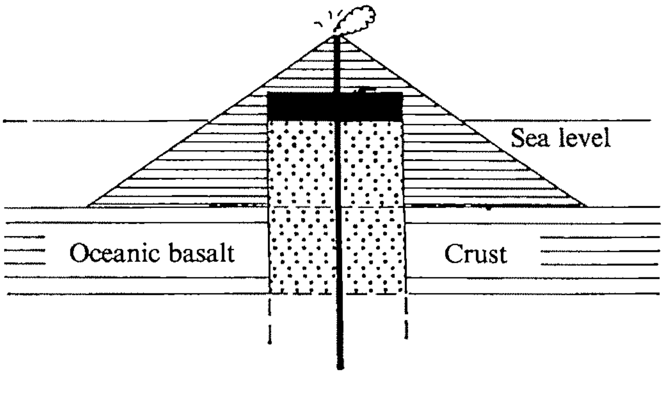
These considerations have led to the development of two criteria of widespread application in studies of basalt genesis and upper mantle motions. If the concentrations of incompatible elements vary greatly in a series of basalts of comparable major element composition, this variation reflects differences in the extent of partial melting at the source region which gave rise to each magma batch. If the ratio of the concentrations of two incompatible trace elements differs between two series of basalts, they have been derived from two different source regions of distinctive chemistry (partly characterised by the ratios of the incompatible trace elements).

These criteria have been critical in underpinning the hypothesis of the existence of hot fertile plumes of upper mantle material rising beneath volcanic 'hot spots'[4]. Their application carries with it the implicit assumption that there has been little fractional crystallisation of the basalts near the surface, and that little chemical change, particularly of the trace element ratios, can have occurred in those near-surface processes. This view apparently conflicts with a wealth of observational and experimental data which indicate that most erupted basalts have been thoroughly modified in composition during near-surface processes[5–9].

The evolution of basaltic magmas by equilibrium or fractional crystallisation in closed systems provides only one model of what could occur in near-surface magma chambers. A slightly more sophisticated model has recently been explored in which the magma in a high-level chamber undergoes continuous fractional crystallisation, but is periodically fed with a new batch of the parental magma from depth. In this model, the arrival of this new magma batch is assumed to displace a sample of the residual liquid from the chamber as a lava flow. The rest of the residual liquid and the new batch of parental magma then mix, and the fractionation process continues.

Many central volcanoes seem to have a core of gabbroic cumulates extending to great depth[10–17]. In the case of oceanic island volcanoes it is manifest that much of what is now plutonic gabbro must have replaced what was previously basalt which had erupted into and had the opportunity to react with seawater. A thick portion of the oceanic crust formed at the ridges is composed of gabbro which may largely have replaced previously erupted basalts. As a refinement to the simplest model, provision has been made to consider the possible effects which are due to the digestion of previously erupted basalts

Fig. 2 Model of the evolution of an oceanic island volcano. The active magma column and differentiating chamber are shown in black. The advancing magma chamber has digested all the previously erupted basalts in the stippled column, replacing them with cumulates. Incorporation of basalts altered by contact with seawater has contaminated the isotopic ratios of later erupted basalts. Because the cumulate pile is built up below the active and advancing magma chamber, this may be a relatively thin sheet of liquid at the cumulate–roof interface. There need never have been a magma chamber of the size of the ultimate gabbro pile.



(Fig. 2). This adds a process akin to zone refining on to the other effects. (This zone refining, however, operates within the fertile ground of previously erupted basalt rather than in the less promising ground of upper mantle peridotites).

Let X = mass fraction of cumulate formed from the magma in each cycle
Let Y = mass fraction of the original liquid before this fractionation which is extracted as a lava flow at the end of each cycle
Let Z = mass of parental magma added in each cycle (expressed as an absolute quantity relative to the initial size of the magma chamber)
Let C_0 = the initial concentration of an element in the parental liquid
Let $C_B{}^S$ = concentration of element in the residual liquid of the fractionation cycle, in the steady state.
Let $D = \dfrac{\text{concentration of element in crystal extract}}{\text{concentration of element in liquid}}$
Let C_Q = concentration of element in the material digested by the magma chamber
Let q = fraction of the total cumulate X fraction, which has been precipitated in order to provide the energy necessary to digest a mass fraction Q of previously erupted basalt. It has been assumed that $Q = qX$ and that $(1-q)X$ represents fractionation caused by conductive and other energy losses.

The following relationship may then be derived. (Full details of these derivations will be given in a longer communication which is in preparation.)

The steady-state mass, M, of the magma chamber is given by $Z/(X-qX+Y)$, M and Z being expressed in any absolute or relative units.

The way in which the potential lava composition, C_B, evolves in successive cycles towards the steady-state composition, $C_B{}^S$, during successive cycles of the addition, mixing and fractionation process, is shown for just one choice of the parameters X, Y, in Fig. 1, curve d ($q = 0$). As X becomes smaller, curve d approximates more closely to c. As X/Y becomes larger, the steady-state composition ($C_B{}^S$) moves to higher values of C^{1q}/C_0.

The concentration of an element in the basalt erupted periodically when the magma has reached a steady state, relative to its concentration in the parental magma, is then given by

$$\left(\frac{C_B{}^S}{C_0}\right) = \frac{\{X[1+q((C_Q/C_0)-1)]+Y\}(1-X)^{D-1}}{1-(1-X-Y)(1-X)^{D-1}} \qquad (1)$$

(provided Z, C_0 remain constant and assuming Rayleigh fractionation).

Figure 3 plots ($C_B{}^S/C_0$) against D for a various values of X, Y, assuming always that $q = 0$. The dotted curves a,a' show the values of D for which one incompatible element will achieve a steady-state concentration 1.5 times greater than that achieved by an element for which $D = 0.1$, 0.2 (lines b,b') for cases where $Y = 0.001$. Much larger (absolute) differences in D are necessary to produce the same effect when D lies closer to unity and separation of elements of similar D value is most effective when $X \gg Y$. When X is large relative to Y, and X, Y, D are all small, it is possible to produce large variations in the ratio of two incompatible elements without large change in the major element composition of the parental magma. The values of X, Y are critical in determining the steady-state concentrations of elements with D very different from unity.

Note that compatible element concentrations ($D \sim 0.4$–2.5) are relatively insensitive to the absolute and relative values of X, Y, especially when X, Y are small and $X > Y$ (Fig. 3).

The X/Y ratio determines the relative concentration achieved by highly incompatible elements, but the absolute values of X and Y become very important as D increases. The larger the values of X, Y, the higher $C_B{}^S/C_0$ of elements for which D $\sim$ 0.3, and the lower $C_B{}^S/C_0$ of elements for which $D > 1$. In other words, the absolute values of X, Y have little effect on $C_B{}^S/C_0$ for incompatible elements, provided X/Y is constant, but greatly influence the concentration of a highly compatible element such as nickel or chromium, the steady-state concentration remaining higher as the absolute values of X, Y decrease.

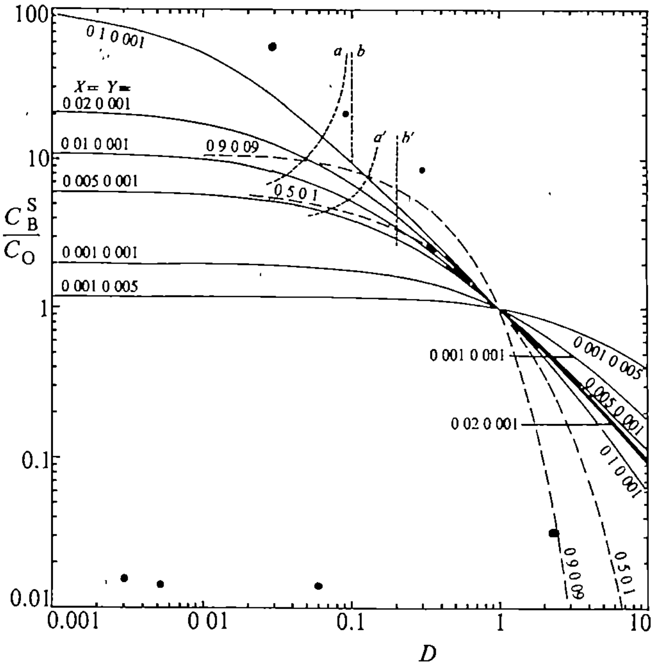


Fig. 3 Variation of the relative concentration of an element ($C_B{}^S/C_0$) as a function of D for a selection of choices of X, Y, ($q = 0$ in equation (1)). At values of D along curves a, a′, the concentration of an incompatible element is 1.5 times that at values of D given on b, b', for all curves with $Y = 0.001$.

$$\frac{C_B{}^S}{C_0} = \frac{(X+Y)(1-X)^{D-1}}{1-(1-X-Y)(1-X)^{D-1}}$$

Further discussion is in the text.

The major geochemical effects hitherto considered to be indicative of small and variable degrees of partial melting or derivation from different upper mantle source regions can also be produced during plausible high-level fractional crystallisation processes for whose operation there is so much independent evidence. This type of fractionation could be effective in exploiting small differences in D (as between isotopes of a single element) to produce small but significant differences in their relative concentrations. (These differences will not be completely removed by the normalisation procedures in standard use unless the differences in D for different isotopes vary linearly with mass.)

The concentration, $C_B{}^S$ of an element in the steady-state liquid is made up of a spectrum of partial concentrations, K_P, each arising from one previous input of parental magma, such that

$$\sum_{P=1}^{P=P} \frac{K_P}{C_B{}^S} = \sum_{P=1}^{P=P} [(1-(1-X-Y)(1-X)^{D-1})] \times$$
$$\times\ [(1-X-Y)(1-X)^{D-1}]^{P-1} \qquad (2)$$

The number of mixing and fractionation cycles, p, for which this summation is equal to 0.5 represents the average residence time (in cycles) of that particular element in the magma chamber. The smaller the values of D, X and Y, the larger p becomes. An incompatible radioactive parent element (for example, Rb) may have been effectively present in the magma for a much longer time than its more compatible decay product (for example, ^{87}Sr).

Returning to equation (1), we can consider the probable effects of the digestion of previously erupted basalts. Setting $q = 0.5$, $C_Q/C_0 = 2$, $X/Y = 1$, $C_B{}^S$ will be 1.25 times as large as it would have been had there been no digestion of basalt. If $q = 0.8$, however, $(C_Q/C_0) = 5$, $X/Y = 3$, $C_B{}^S$ becomes 3.4 times larger than it would otherwise have been, and the contribution of digestion of previously erupted baslts begins to dominate the geochemical effects.

The potential effect of the digestion of previously erupted basalts, whose isotopic ratios have been altered by interaction with seawater, to influence the isotopic ratios in the later basalts to be erupted may be seen by rewriting (1) to obtain the fractional change in the isotopic ratio, Δ, where

$$\Delta = \left\{\left(\frac{{}_1C_B{}'^S}{{}_1C_B{}^S}\right)\left(\frac{{}_2C_B{}^S}{{}_2C_B{}'^S}\right) - 1\right\}$$

and ${}_1C_B{}^S$ = steady-state concentration of isotope 1 if $q = 0$, ${}_1C'_B$ as here = steady-state concentration of isotope 1 if $q \neq 0$, and so on. Assuming no difference in D for the isotopes 1, 2, and setting the ratios of the two isotopes in the altered basalt

$$\frac{{}_1C_Q}{{}_2C_Q} = r\left(\frac{{}_1C_0}{{}_2C_0}\right)$$

then

$$\Delta = \left(\frac{X}{Y}\right)q\left(\frac{{}_2C_Q}{{}_2C_0}\right)(r-1) \bigg/ \left\{\left(\frac{X}{Y}\right)\left[1+q\left(\frac{{}_2C_Q}{{}_2C_0}-1\right)\right]+1\right\} \qquad (3)$$

Δ has been calculated for the values of q, C_Q/C_0 and X/Y discussed above. If $r = 1.0014$ (the fractional change in $^{87}Sr/^{86}Sr$ for average ocean floor basalts deduced by Spooner[18]), then $\Delta \sim 5$ to 13×10^{-4}, which is of the order of size observed between fresh ocean floor basalt and ocean floor basalt and oceanic island theoleiites[19].

Isotopic fractionation (with D values varying non-linearly with mass), larger effective residence times in the magma chamber for an incompatible parent element than for its radiogenic products, and digestion of previously erupted, isotopically altered, basalts could all contribute to changing the isotopic ratios of the basalts erupted at a later stage in the development of oceanic islands. The last of these processes apparently has the greatest potential. It could be that a substantial part of the differences in the isotopic ratios of Sr, Nd and Pb between basalts from hot spots relative to those from mid-ocean ridges may reflect the longevity and comparative geochemical fertility of seawater rather than of any underlying mantle source region.

Contamination by assimilation of crustal material could also occur during the creation of cumulate piles in central volcanoes of continental regions.

In discussing the possible effects of contamination on major or trace elements, or isotopes of elements, several relevant factors must be borne in mind. It is the local average continental crust, or isotopically altered oceanic crust (not seawater itself) which is causing the contamination and which must, therefore, be characterised. The advancing magma chamber will thermally metamorphose the assimilated materials before they are digested; there may be further isotopic exchange with expelled waters at this stage. The assimilation of basalt by basalt magma would create no dramatic change of major element chemistry of the magmas or the cumulates, but the assimilation of crustal material might well do so. The effects of this chemical contamination cannot be simply envisaged, however, because the speeds with which the added elements are removed to the cumulus pile and their steady-state concentrations in the magma are functions of the distribution coefficient for each element. Where the contamination is severe, the bulk distribution coefficients could themselves be modified due to the change in the nature or proportions of the minerals precipitating.

Thus far the effects of this model of magmatic evolution on trace elements, for which $D \sim$ constant have been treated. For the major components, D is not constant, changing progressively with crystallisation of a phase within any one equilibrium (for example, olivine + liquid) and changing abruptly

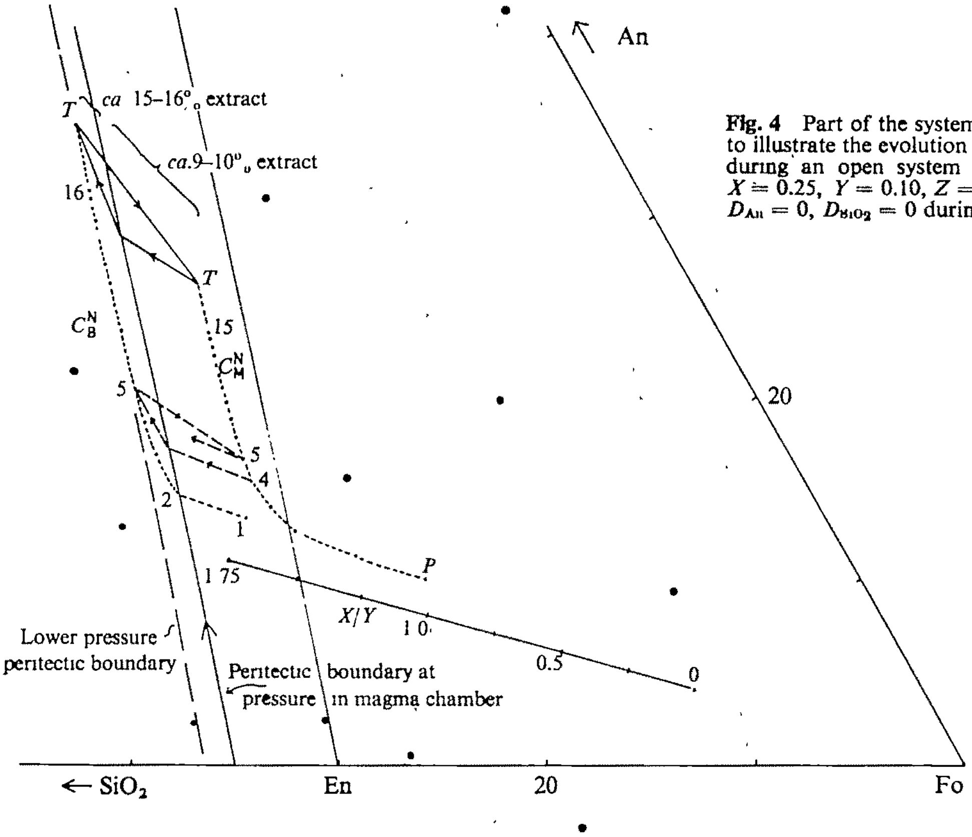


Fig. 4 Part of the system forsterite–silica–anorthite (simplified) to illustrate the evolution of the major components of the liquid during an open system fractionation process $C_0{}^N = C_0 = P$, $X = 0.25$, $Y = 0.10$, $Z = 1.0$, D_{Fo} = variable, D_{En} = variable, $D_{An} = 0$, $D_{SiO_2} = 0$ during Fo crystallisation only (discussed in the text).

whenever there is a change of equilibrium (for example, to olivine + pyroxene + liquid). The possible effects here are difficult to convert to mathematical models, but can be handled by graphical means. Figure 4 shows where the steady-state liquid composition will lie for two particular choices of parent liquid composition, and how one of them evolves during successive mixing and fractionation cycles to reach that composition.

With parental magma composition O, (Fig. 4), the steady-state liquid composition may lie anywhere along the line from forsterite through O, depending upon the ratio of X, the amount of crystals extracted, to Y, the amount of magma erupted.

With parental magma P the amount of fractionation supposed is sufficient for the residual liquids in successive cycles to evolve beyond the cotectic between pyroxene and forsterite. The evolution of the liquid towards the steady-state concentration has been traced graphically for one choice of X, Y. The actual evolution of the liquid between the fourth mixed magma (C_M), the fifth residual magma C_B, and the fifth new mixed magma (C_M) is illustrated by dashed lines. The loci (but not the path) of the evolution of liquids C_B, C_M to their steady-state compositions, T, are shown by dotted lines. The steady-state recycling by repeated fractionation and mixing is shown by a triangle of solid lines. The loci C_B, C_M approach each other as X becomes smaller. The steady-state compositions move further from O, P as X increases relative to Y.

The important general principles to be derived from Fig. 4 are that the steady-state liquid composition could be arrested within the liquidus field of a single crystalline phase (for example, olivine or pyroxene), or on a cotectic where two or more phases are precipitating together. In certain circumstances, such as those depicted in Fig. 4, which are relevant to the crystallisation of olivine from natural basalts at low pressures, steady-state residual liquid compositions are constrained to lie close to those of liquids which are in reaction relationship with olivine. This result contradicts the general rule in closed system fractional crystallisation, that residual liquids will depart in composition relatively rapidly from a peritectic relationship (see Fig. 4). To add to the possibly misleading aspects of the effect shown in Fig. 4, any drop in pressure which accompanies the eruption of a liquid from the magma chamber to the surface will expand the limits of the olivine liquidus field (Fig. 4), tending to place the locus of the peritectic liquids at low pressure even closer to the liquid compositions generated at a slightly higher pressure.

Summarising, in this open-system fractional crystallisation process, a near-surface magma chamber is fed regularly with batches of parental magma which mingle with the liquid already there. The mixed magmas undergo continuous fractional crystallization. The steady-state lavas which may be extracted from time to time will then show the following characteristics: (1) Uniformity of composition, provided the thermal insulation of the magma chamber, the rate of supply, the amount and nature of assimilation and the composition of the parental magma are maintained constant. (2) Large differences of composition between the lava and the parental magma with respect to major and trace element concentrations and ratios. (3) Strong control of liquid composition by the low pressure phase equilibria. The steady-state liquid composition may be arrested within the liquidus field of one crystalline species only (specifically, that which would be the liquidus phase in the parental magma). It is, however, more likely to be located on a cotectic where two or more crystalline species precipitate together, or very close to a peritectic where one (or more) crystalline species is in reaction relationship with the liquid while others precipitate.

Steady-state lavas developed from the same parental magma but in magma chambers with different characteristics of heat loss and supply rate may show the further characteristics:

(4) Large variations in the concentrations of incompatible elements (for example, the large-ion, lithophile elements) with litttle change in the concentrations of the more compatible major elements, and little variation in the iron: magnesium ratio.

(5) Significant variations in the ratio of two incompatible elements.

If the magma chamber advances ahead of the growing column of cumulates within the growing volcano in part by digestion of previously erupted basalts, then:

(6) Enrichment factors mentioned in (4) may be greatly enhanced in the later products of the volcano.

(7) Isotopic characteristics of the previously erupted basalts, which may have been altered by contact with sea water, will influence the isotopic characteristics of the later products of the volcano. Higher ratios of the radiogenic to non-radiogenic isotopes of Sr, Pb, Nd in the lavas erupted from large central oceanic island volcanoes than in ocean floor basalts may partly reflect the chemical characteristics of sea water. This process can explain the difference in strontium isotope ratios observed[7] between oceanic island and oceanic floor basalts quantitatively as shown above. Data are lacking at present for isotopic ratios of Pb, Nd in the average altered oceanic basalt section, hence this part of the prediction cannot yet be tested.

Provided X, Y are small,

(8) Highly compatible elements (for example, Ni, Cr) may show relatively high concentrations despite the effects noted in (2), (4).

(9) The mass of the magma chamber is large relative to the periodic batches of parental magma added to it, and the composition of the steady-state magma will be insensitive to short-term random fluctuations in the mass or composition of the added batches.

Lastly,

(10) There is no requirement for the magma chamber to be large. The whole ocean floor gabbro sequence might be laid down from a magma sheet never more than a few tens of metres thick, as might many cumulus piles in central volcanoes.

As a general comment one may note that few of these features are anticipated as a result of closed system fractional crystallisation processes.

The geochemical features noted under (1), the second sentence of (3), (4), (5), (6), (7) or (8) can therefore only be used in support of claims that particular basalts are primary, have been derived by different degrees of partial melting of some source region, or have been derived from fundamentally different source regions, if the model of volcanic plumbing discussed in this paper can be specifically excluded. Because of the feature mentioned in (2), overlooking the effects of high-level fractionation which are possible results of this model will result in misleading conclusions about the chemistry of the source regions and the processes which have affected them. The hallmark which indicates that lavas may have evolved in this type of process is set out in (3)—often the lavas will be porphyritic, but in general will be close to saturation at low pressure with two or more phenocryst species.

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An oxygen-isotope climatic record from the Devon Island ice cap, arctic Canada

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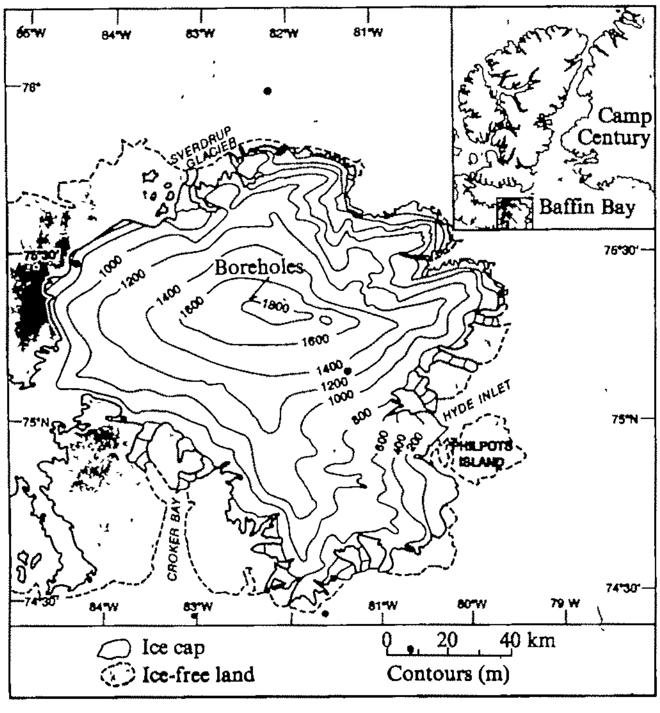
Isotope measurements on two adjacent cores through the Devon Island ice cap provide a well-dated climatic record for the past 5000 yr. Fluctuations in annual values include much 'noise', and ice flow over a rough bed produces distortions in the lowest 5% of core which covers roughly 120,000 yr. Comparison with the Camp Century, Greenland, record helps to separate climatic changes from changes in ice thickness or flow pattern.

SINCE the $^{18}O/^{16}O$ ratio in polar snow depends on its temperature of formation[1], deep cores from polar ice sheets contain a record of past temperatures. Records covering all or part of the Wisconsin Glaciation have been obtained from Greenland and Antarctica[2–5]. We report here on two cores from the Devon Island ice cap, arctic Canada. These cover the same period as the Camp Century, Greenland, core, namely about 125,000 yr, but because the ice more than 10,000 yr old is compressed into a few metres near the glacier bed, the record is less detailed.

The $^{18}O/^{16}O$ ratio is expressed as the fractional difference between the ratio in the sample to the ratio in 'standard mean ocean water'. It is denoted by δ and measured in ‰. In polar snow δ is negative; the more negative, the lower the temperature. Correlations between δ and temperature must be made with caution, however, because δ will also change if the source area of precipitation changes, if the ice thickness changes with time or if the ice at depth originates upslope from the drill site[2]. Drilling near an ice divide, as in the present study, eliminates the last problem.

Fig. 1 Map showing location of boreholes on the Devon Island ice cap and its position relative to Camp Century, Greenland.



The ice cap and boreholes

Figure 1 shows the ice cap and borehole site some 600 km from Camp Century on the opposite side of Baffin Bay. The boreholes, Holes 72 and 73, are 27 m apart on the same flow line about 900 m north of the ice divide. Hole 73 is upslope from Hole 72. The holes reached bedrock at depths of 298.9 m (Hole 72) and 299.4 m (Hole 73). The ice–bedrock contact was sharp; the lowest metre of each core contained a few small dirt particles, probably of wind-blown origin, but there was no basal moraine in the ice. The temperature at the interface was −18.4 °C. Annual precipitation is 0.22 m water equivalent and the mean annual air temperature is about −23 °C. In most summers, a little surface snow melts and refreezes in the snowpack. This, combined with the relatively low precipitation rate, means that seasonal variations in δ are eliminated in a few years.

Dating the ice

For a palaeoclimatic record to be of any value, the age of the ice at each depth must be known. Age t at depth y is calculated by the formula

$$t = \int_0^y v^{-1}\,\mathrm{d}y$$

where $v(y)$ is the component of ice velocity perpendicular to the surface. In previous studies, various functional relationships between v and y have been assumed[6,7]. In the present case, v was measured at various depths in the borehole[8] and the integral evaluated numerically. Although a measured relation should be better than a theoretical one, the measured values must be assumed typical of past values. This implies that the ice cap has been in a steady state (no change in precipitation rate, ice thickness or temperature) for the time period covered by the core. This may be a reasonable approximation for a period of a few thousand years but not for one extending into the last glaciation. At any depth in a steady-state ice cap, the value of v, expressed as the distance an ice particle moves downward in 1 yr, should be equal to the thickness of an annual ice layer, a quantity that can be determined from seasonal variations in concentration of microparticles in the core[9]. Thirteen such measurements (10-yr means) in the youngest 2,100 yr of core checked satisfactorily.

The time scale was further checked by absolute dating of the ice at four points by the ^{32}Si method[10] and at another four points by ^{14}C obtained by down-borehole extraction of CO_2 from air bubbles in the ice[11].

Figure 2 shows the time scale and absolute dates. The curve is within 1 s.e. of each of the ^{32}Si dates. The discrepancies that exist may be real rather than statistical because similar differences at about the same ages have been observed in two Greenland cores. This suggests that the production rate of ^{32}Si in the atmosphere may vary with time. The curve is also within 1 s.e. of two of the four ^{14}C dates and within 2 s.e. of another. The discrepancy of the youngest date is very large, however. We conclude that this ^{14}C date is wrong, probably because the sample was contaminated by fossil CO_2. A ^{13}C/^{12}C analysis supports this idea. The standard error of the calculated ages, based on the standard error of measurement of v, is about 6% for the first 1,500 yr and increases to about 20% at 5,000 yr. The precision of the ages is probably better than this because the curve agrees with most of the absolute dates. We therefore believe that the record for the past 5,000 yr is well dated.

Comparison of adjacent cores

Having two boreholes 27 m apart enabled the variation in δ over short distances to be measured. To check the repeatability of the mass spectrometer measurements, 400 samples from one core were measured twice. The correlation coefficient between measurements was 0.991; thus instrumental inaccuracies are negligible. Next, 450 samples, each of the calculated annual layer thickness, were cut from each core. The δ values were compared in equivalent segments corresponding to 50 calculated annual layers. The correlation varied between 0.45 and 0.72. These relatively low values show that much of the variation in annual values of δ is 'noise'. Part of it probably results from variations in snow accumulation over short distances so that samples cut to the mean annual layer thickness will contain varying amounts of summer and winter snow; the remainder probably results from variations in the amount of meltwater percolation.

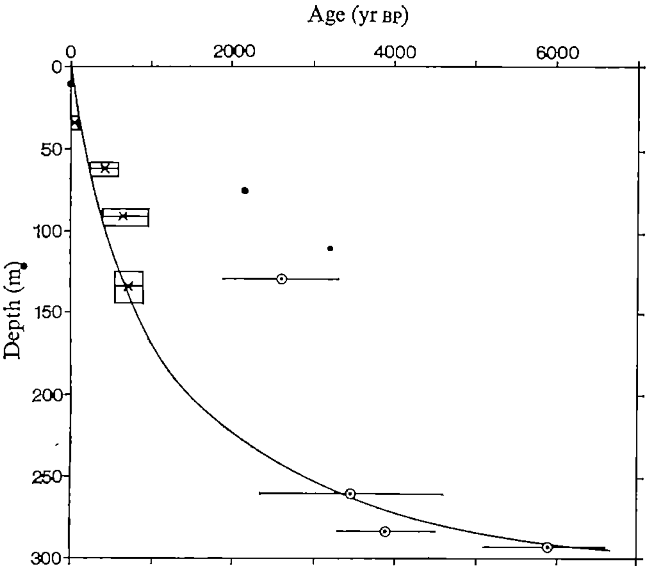


Fig. 2 Time scale for the core, based on measured vertical velocity, and absolute dates. The vertical lines beside the ^{32}Si dates indicate the depth interval over which each sample was taken. Each ^{14}C sample covers a depth of 1.5 m X, ^{32}Si date; ⊙, ^{14}C date. Bars are 1 s.e on each side of mean.

The isotope profiles from the two boreholes closely resemble each other except near the base of the ice. The correlation between 50-yr mean δ values is 0.965 between the surface and 13 m above the bed, but only 0.449 between 13 and 5 m. The correlation decreases because bumps and hollows in the bedrock disturb the regular flow pattern in the ice immediately above.

Figure 3 shows the lowest 5 m of each record. Although the profiles show many of the same features, gaps in each are apparent. No gap is more than 50 mm long and construction of a composite record, described later, suggests that 25–30% of each record is missing. This assumes that no part of the record is missing from both cores, which may not be true. The extremely steep δ gradients at certain points may be additional evidence for gaps. For example, the feature at 2.6 m in both cores corresponds to a change of 9‰ in about 30 mm of core and the feature at 2.3 m in core 72 to a change of 5‰ in 10 mm. Because molecular diffusion reduces δ gradients, these values must have been greater in the past. We consider it most unlikely that such gradients represent climatic effects; a recent tectonic origin seems more probable. We emphasise that such gaps do not represent missing core sections; a complete core was recovered from each borehole. Nor can the gaps result from ablation; there is no ablation at the drill site at present and most gaps occur in the Wisconsin part of the record when temperatures were much lower than present.

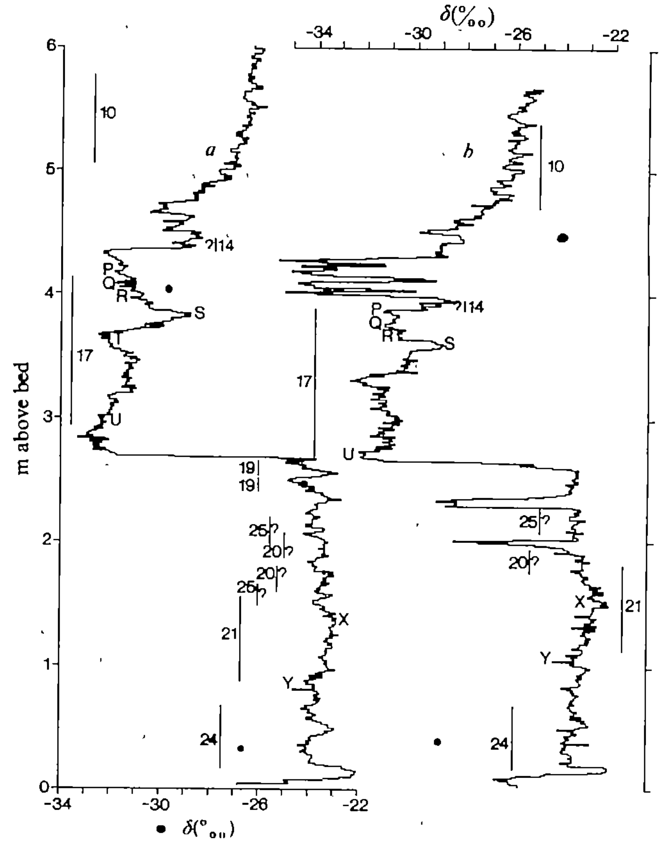


Fig. 3 Values of δ, for samples 10 mm long (*a*, core 73; *b*, core 72), against distance above bedrock for lowest few m of each core. Sections and features marked with the same number or letter are considered to be equivalent. The 'cold peak' at 2 m in Core 72 is doubtful; it may result from a sampling error. Gaps up to 50 mm long are apparent in both cores.

Variations in vertical strain rate, and thus in annual layer thickness, over short horizontal distances, can probably explain most of the disturbances in the lowest part of the record. The strain rate is expected to vary as the ice moves over an uneven bed; thinning and 'pinching out' of horizontal layers of bubbles observed in vertical sections of core, provide evidence of such variations. The apparent gaps in the record may be highly compressed regions where the isotope variations have been smoothed out by diffusion. Faulting or movement along narrow zones of intense shear provide another possible explanation of the gaps. Although these processes might occur near bedrock bumps and hollows, the cores show no definite crystallographic evidence for them. Large changes in grain size occur over short vertical distances but there are no thin bands of very fine-grained ice characteristic of shear planes. Folds might also be expected to form. These would produce inverted sections of record; segments 25 and 20 in core 73 (Fig. 3) may perhaps show this. Again, however, there is no crystallographic evidence of folding.

Climate and ice thickness changes 0–5,250 BP

The point, 13 m above the bed, dated at 5,250 BP where the correlation between the two records starts to break down corresponds closely to the limit of the accurate time scale. We therefore present the results in two parts: (a) the period 0–5,250 BP and (b) the section from 5,250 BP to the bottom of the core (dated at roughly 125,000 BP by comparison with the Camp Century record) where we do not assign dates and discuss only major features. Except for the lowest 5 m, plotted δ values are an average of the values for the two cores. The estimated standard error of an annual value in the combined record is 0.5‰.

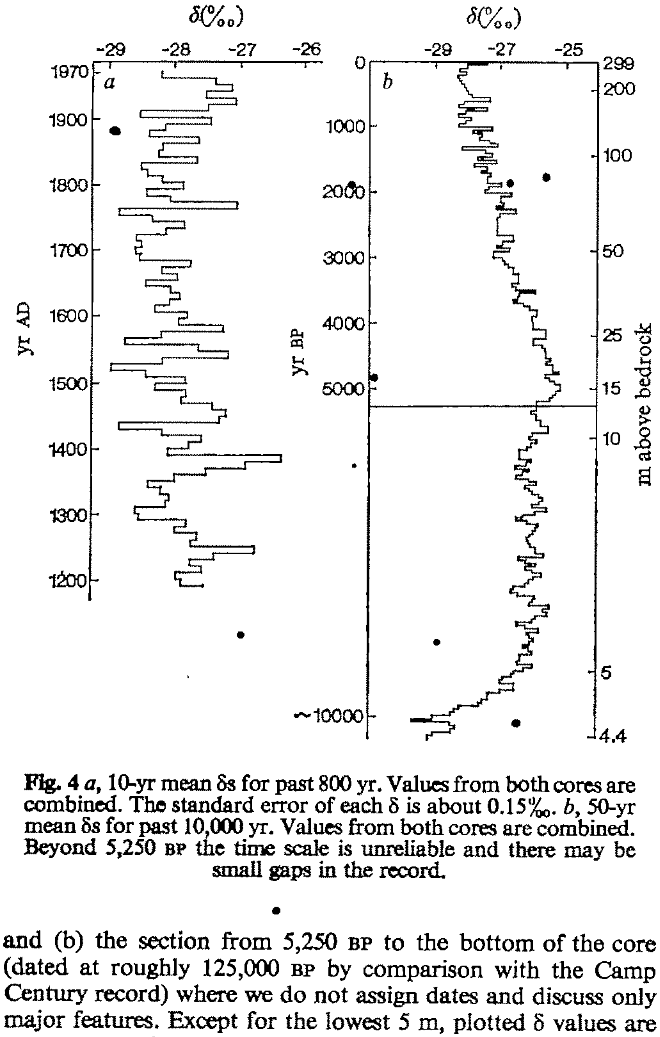


Fig. 4 *a*, 10-yr mean δs for past 800 yr. Values from both cores are combined. The standard error of each δ is about 0.15‰. *b*, 50-yr mean δs for past 10,000 yr. Values from both cores are combined. Beyond 5,250 BP the time scale is unreliable and there may be small gaps in the record.

Figure 4*a* shows 10-yr mean δ values from AD 1200 to present. Prominent features are brief warm periods with peaks at 1240 and 1380, cold peaks at 1430, 1520 and 1560, the 'Little Ice Age' continuously cold from 1680 to 1730 and with another temperature minimum at 1760, a pronounced warming at about 1910 with relatively warm temperatures until about 1960 and a marked cooling thereafter. The Camp Century record[2] also shows most of these features, although some dates are different; in particular, the coldest part of the Little Ice Age occurs at about 1660. Such differences are not surprising; the Little Ice Age does not even appear in the period 1500–1700 in the record from Crête in central Greenland[12].

Figure 4*b* shows 50-yr mean δ values for the past 10,000 yr. The warmest part of the Climatic Optimum occurred on Devon Island at about 5,000±800 BP. The date 5,000 BP is more likely to be too old than too young because a value greater than 5060 BP would be outside the limit of 2 s.e. of the ^{14}C date (3860±600 BP) at this point.

The record shows a long-term decrease in δ from 5,000 BP to present; this does not appear in the Camp Century record[2,4]. Figure 5 shows this feature. In *a*, curve 1 is the last 5,000 yr of the Camp Century record, smoothed by a 5,000-yr low-pass filter applied to the last 10,000 yr of the record. The time scale is that calculated from the Dansgaard–Johnsen flow model[6]. Curve 3 is a similar curve for Devon Island. Part of the difference between the two arises because the deeper layers in the Camp Century core were formed at higher elevations inland from the station. Curve 2 is the trend corrected for this effect, based on the present surface configuration and ice velocity at Camp Century and the assumption that a 100-m increase in elevation changes δ by −0.62‰ (ref. 13). The Devon record needs no correction because the boreholes are near the ice divide, provided that the divide has not migrated during the past 5,000 yr. Even after the flow correction is made, the change in δ at Camp Century is less than at Devon Island (0.6 compared with 2.2‰). On the other hand, the shorter-period trends in δ at the two stations seem to be of about the same size (Figs 5*b* and *c*).

Although it is possible that the long term cooling has been much greater at Devon Island than at Camp Century, changes in ice thickness provide a more likely explanation of the difference. Koerner[14] has shown that the percentage of ice layers in the Devon cores is highly correlated with the ice cap's mass balance. He concluded that accumulation balanced ablation from about AD 1300 to 1860 and that the mass balance has been slightly negative since then. Thus during the Climatic Optimum, when δ was more than 2‰ less negative than at present, the ice cap was probably smaller and thinner than it is now. The change in δ at Camp Century would correspond to a cooling of roughly 1°. The additional 1.6‰ at Devon Island would correspond to a thickness change of about 250 m. This seems excessive in view of the broad agreement between the time scale calculated from the present vertical velocity and the ^{14}C dates. Thus the cooling trend over the past 5,000 yr has probably been more than 1°. If so, it has been partly obscured in the Camp Century record by thinning of the ice there since the Climatic Optimum[15]. Other interpretations of the difference between the long term trends in δ are of course possible. Moreover, we have ignored the possibility that δ has been affected by changes in atmospheric circulation patterns.

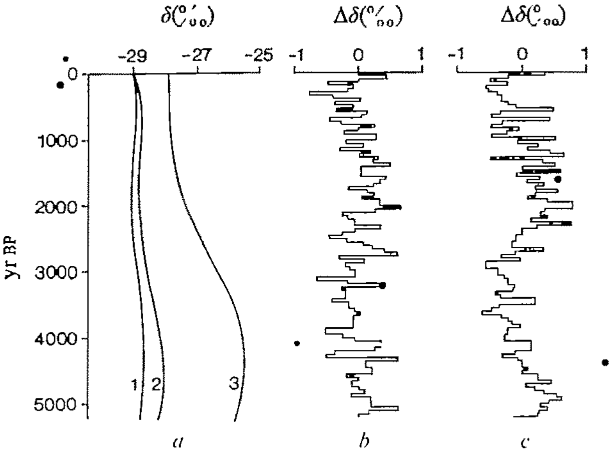


Fig. 5 *a*, Trend lines in δ for past 5,000 yr (1) for Camp Century, (2) for Camp Century corrected for ice flow from up slope, (3) for Devon Island. *b*, residual from trend for Camp Century. *c*, residual from trend for Devon Island. The Camp Century time scale is that of ref. 6.

The glaciation record

In Fig. 6*a*, the pre-Holocene record is a speculative reconstruction using the lowest few metres of both cores. The following rules were used in the construction: (1) Where segments of the two cores seem to be roughly equivalent the longer of the two is used. (2) Any repeated sections are used only once. (3) Where there is a large gap in one core, the record from the other core is used and placed in the position consistent with adjacent common equivalent segments. (4) It is assumed that no segment is missing from both cores. (As we estimate, however, that each core lacks 25–30 % of the record, it is likely that 6–9 % of the combined record is missing.) The record broadly resembles that of Camp Century (Fig. 6*b*): a cold period between about 10,000 and 65,000 BP (Camp Century dates) preceded by a warm period of roughly the same duration.

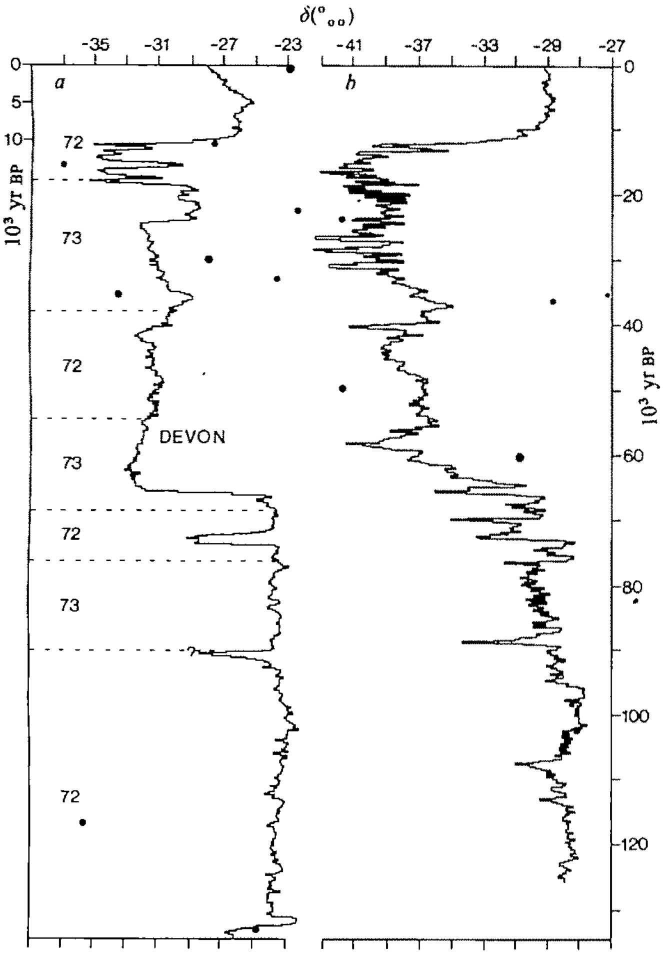


Fig. 6 *a*, Profile of δ for Devon Island. The Holocene section is that of Fig. 4*b*. The remaining section was obtained by combining the records from the lowest few metres of the two cores (See Fig. 3). While more nearly complete than either record, the combined one probably still has gaps. *b*, Profile of δ for Camp Century, Greenland[8].

A major difference between the stations is in the change in δ at the end of the Wisconsin Glaciation about 10,000 BP. The change at Camp Century is 11‰, at Devon Island 8 %, and 5 or 6‰ in Antarctica[4,5]. While the temperature change at the end of the Wisconsin may have been greater at Camp Century than elsewhere, a more likely explanation is that the Wisconsin ice in the Camp Century core originated at a much higher elevation than that of the present station. Measurements of total gas content of the ice, a quantity negatively correlated with the elevation at which the ice formed, seem to support this view and suggest that the Wisconsin ice originated at about 1,300 m higher than the Holocene ice[16]. Such a difference is more than enough to account for the exceptionally large change in δ. The most likely explanation is that the Wisconsin ice originated near the main divide of the Greenland ice sheet, whereas the Holocene ice originates within 50 km of the station[17]. The reason for such a drastic change in flow pattern is unclear; it may be related to the rise in sea level at the end of the Glaciation.

The difference of 8‰ between Wisconsin and Holocene ice at Devon Island should not be interpreted as purely a climatic effect because the ice probably thinned at the end of the Glaciation. A rough estimate of the difference in thickness, based on standard ice cap profiles and the assumption that the ice margin at the late-Wisconsin maximum was in the position shown in the map of Prest[18], is 150 m. Because this increased load would depress the bedrock, the increase in surface elevation would be 100 m which would change δ by about 0.6‰ (ref. 13). An additional change of 0.6‰ would result from the 100 m lowering of sea level during the Glaciation. Thus the climatic effect is perhaps about 7‰. During the relatively warm period in the Glaciation δ was more negative than at present. Thus this period was an interstadial rather than an interglacial.

Oldest part of record

Except for short intervals, values of δ in the lowest 3 m of the Devon record are 0.5–3‰ less negative than at the peak of the Climatic Optimum. This segment seems to correspond to the part of the Camp Century record estimated to extend from 65,000 to 120,000 BP (Fig. 6). Values of δ are less negative than in the Climatic Optimum in much of this part of the Camp Century record also. The obvious interpretation is that, during this period, temperatures were higher and the ice thinner than at present, except for relatively brief intervals in which temperatures fell to glacial values. An alternative explanation, however, of the relatively high values of δ might be that, during this period, the major source area of precipitation on the ice caps was different from the present one. Two independent lines of evidence suggest that world sea level was below present from 65,000 to 120,000 BP (refs 19,20); thus glaciation in many parts of the world must have been more extensive than it is now. Moreover, studies of pollen[21] and soil[22] in northern Europe indicate that glacial conditions began there about 110,000 BP, although there is evidence that North Atlantic water remained relatively warm until about 70,000 BP (ref. 23). For these reasons we think that the high δ values probably indicate a different source of precipitation rather than a less extensive ice cover.

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letters to nature

Inverse Compton γ rays from Cyg X-3?

THE detection of ~100-MeV γ rays from Cyg X-3 has been reported from recently published SAS-2 data (R. C. Lamb *et al.*[17]). The γ-ray luminosity is $\sim 10^{37}$ erg s^{-1} assuming the source is ~10 kpc distant. Cyg X-3 is well known as an X-ray and infrared source, and a source of non-thermal radio outbursts[1] ($L_x \sim 10^{38}$, $L_{IR} \sim 10^{37}$, $L_{radio} \sim 10^{33}$–10^{35} erg s^{-1}). The infrared, X-ray and γ-ray fluxes all display a 4.8-h modulation in phase with one another[1], which we shall presume to be attributable to binary motion[2,3]. We note here that inverse Compton scattering of ~100 MeV relativistic electrons, in a region between the X-ray source and its companion, by the plentiful X rays, provides a mechanism for the γ-ray emission and its observed modulation.

Modulated γ rays are most efficiently produced by injecting the relativistic electrons in such a region for the following reasons. The γ rays cannot be created too close to the X-ray source ($R_x \sim 10^{10}$ cm), or they would be destroyed by pair creation in γ-ray–X-ray collisions. Creation outside the binary orbit would not allow sufficient modulation and would, if the emission is by inverse Compton scattering, be inefficient, as adiabatic losses would dominate the cooling of the relativistic electrons. It seems likely that the source of X-ray modulation is primarily electron scattering; the scattering optical depth must be of order unity to account for the observed amplitude of X-ray modulation (ref. 2 and references therein). But if the γ rays came from a region near the X-ray source (but $R_x \gtrsim 10^{10}$ cm), they could not be modulated by electron scattering because the Klein–Nishina cross section is greatly suppressed at γ-ray energies.

We thus have a picture of a close binary system with a cloud of relativistic electrons close to and on the X-ray source side of the primary. Modulation of the γ rays, generated by inverse Compton scattering, in phase with the X rays, arises naturally as γ rays will be most strongly emitted in backscattering X rays (compare ref. 4). The γ-ray power emitted towards the observer is approximately $\propto (1 - \sin i \cos \varphi)^2$, where i is the orbital inclination and φ is the observed phase ($\varphi = 0$ is X-ray minimum). Additional γ-ray modulation could come from partial eclipse of the emitting region as it is closer to the primary than the X-ray source, which is not eclipsed. The depth of γ-ray modulation depends upon the position and extent of the electron cloud.

What is the required source ($\gtrsim 10^{37}$ erg s^{-1}) of relativistic electrons? For a lower limit on the timescale for changes in the 4.8-h period of $\sim 10^5$ yr (ref. 5), it seems improbable that this energy could come from the orbit or the spin of the primary. The X rays themselves could drive winds at $v \sim v_{escape} \sim 10^3$ km s^{-1} from the primary which could be shocked, but the efficiency of thus producing relativistic particles is then necessarily high ($\gtrsim 10\%$). The only remaining energy source seems to be the spin of a neutron star/X-ray source. An attractive possibility is that the source of X rays and relativistic particles is a young pulsar as suggested by Lamb *et al.*[17]. The X rays might then exhibit a $\lesssim$30-ms pulsar period. This would obviate the need for any mass transfer.

The model may be developed a little further to account for the infrared observations. If the relativistic electrons are injected in a region magnetised to say $B \sim 500$ G, then a distribution $dn_e/dE \propto E^{-4}$ for $E \geq 100$ MeV can produce the observed infrared flux. The infrared photons would be free–free absorbed for $\lambda \gtrsim 2$ μm accounting for the rising spectrum[6]. The observed modulation[1] can be attributed to electron scattering similar to the X rays, but with smaller amplitude as the infrared source is closer to the primary. Unfortunately, Faraday rotation in the wind would probably completely depolarise this source of infrared. We may predict, however, that if the source of γ-ray and infrared emission in this model is correct, then γ-ray and infrared variability (non-binary) should be correlated. Alternatively, if the infrared comes from a circum-binary shell as proposed by Milgrom[7], such correlation may not be expected.

We also note that inverse Compton scattering by the stellar photons (which mostly come from incident X rays re-radiated in the ultraviolet band) and by the infrared photons in this model could account for the hard X-ray tail[8], which would again be modulated by electron scattering. If this is correct, then the infrared and hard X-ray fluxes should show similar 4.8-h modulation and similar variability on other timescales.

Cyg X-3 is a spectacular emitter of polarised radio waves[9–12]. If, as seems likely, the emission is synchrotron radiation, this provides independent evidence for the acceleration of relativistic electrons in this system. Radio emission is then detectable, however, at gigahertz frequencies only when the radio-emitting electron cloud has dimensions $\sim 10^{14}$ cm (ref. 11). Such a cloud cannot be closely related to the source of γ rays and infrared we have proposed. The radio cloud could hardly produce 4.8-h modulated γ rays, and the γ-ray emitting cloud could not expand to 10^{14} cm without suffering catastrophic energy losses. It is interesting, however, that the densities and field strengths ($n_e \sim 10^7$ cm^{-3}, $B \sim 0.1$ G) proposed in a shock-wave model[11] for the radio cloud are compatible with those inferred above within the orbit if the field is toroidal ($B \propto r^{-1}$) and $n \propto r^{-2}$.

Finally we note that Circinus X-1, which has an X-ray period of 16.6 d (ref. 13), is also an infrared[14,15] and non-thermal radio source[16]. Its light curve may be interpreted as an extreme example of the asymmetry present in Cyg X-3. A γ-ray study of this source would clearly be of interest.

Further γ-ray observation of Cyg X-3, and observations of Circinus X-1, to better define the 4.8-h light curve, is needed. Correlated γ-ray, hard X-ray, infrared, and radio observations will be extremely useful in defining the site(s) and source(s) of relativistic electron injection in this system.

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Amplification of cosmic Cerenkov radiation

EJECTION of relativistic or ultra-relativistic electrons is used in most quasar models to explain the very high luminosities of these objects. The velocity distribution of matter is taken as[4]

$$F(r) = F_0\,\gamma_0^{-3/2} \tag{1}$$

where

$$F(r) = \frac{1}{M}\int_r^{\infty} 4\pi\rho r^2 \mathrm{d}r$$

is the mass fraction external to the radius r; $F_0 \approx 10^6$; ρ is the density and the Lorentz factor is $\gamma_0 = (1-u^2/c^2)^{-1/2}$; u and c are velocities of the particle and the light, respectively. The observed luminosities are then explained on the basis of inverse Compton scattering of synchrotron radiation to the optical frequencies. In the radio frequency range the observed emission spectrum shows polarisation and also follows the power law, suggesting a synchrotron emission mechanism supporting these models, which essentially lead to the existence of finite magnetic fields in the quasars[1,2]. In these conditions, some Cerenkov radiation is also produced[4,5]. We suggest here that this radiation is amplified, and may account for the observed luminosity of quasars.

The energy density of magnetic field $B^2/8\pi$ is taken to be greater than the energy of the relativistic particles[3–5] and the non-relativistic electron density, n_0, is $< 10^8$ cm^{-3}. The emission of synchotron radiation at 10^8-Hz frequency, requires a magnetic field of the order of 10^2 G. These parameters also satisfy conditions for Cerenkov emissions and may explain observed optical luminosities due to inverse Compton scattering[4,5]. Explanations based purely on the Cerenkov emissions, however, need linear dimensions of the order 10^{14}–10^{16} cm, which are more or less equal to quasar dimensions. Therefore, the assumptions regarding conditions for 'Cerenkov emission' in these regions are rather doubtful. Moreover, the relativistic particle distribution of the type given by equation (1) can also amplify the Cerenkov radiation, which seems to be important.

We suggest here, therefore, that the wave emitted by the relativistic particle in the Cerenkov mode might undergo amplification by Landau mechanism. It is important to point out that the Cerenkov emission and its amplification are two entirely different processes; the former is due to individual particles, whereas the latter is a collective phenomenon. Therefore, in the magnetoplasma the plasma of sharply defined energy will not amplify its own Cerenkov radiation. In the presence of a distribution of energetic particles, such processes may explain the very low frequency emission in the magnetosphere[6–8]. In the case of quasars the energetic particle distribution follows equation (1) and seems more suitable for amplification of the Cerenkov radiation, as discussed below.

A relativistic electron moving with velocity **v**, in a medium with refractive index $\mu > 1$, emits energy ε_ω[4]

$$\varepsilon_\omega = \frac{2e^2}{c^2}\omega\left(1-\frac{c^2}{\mu^2 v^2}\right)\text{erg s}^{-1}\,\text{Hz}^{-1} \tag{2}$$

in the frequency band near ω, where e is the electron charge. These waves, for propagation at angle θ to the magnetic field **B**, satisfy the dispersion relation[6]

$$\mu^2 = \frac{c^2k^2}{\omega^2} = \frac{\omega_p{}^2(\Omega_e+\omega)}{\omega\Omega_e{}^2\cos\theta} \tag{3}$$

for frequencies $\omega_p, \Omega_e > \omega \gg \Omega_i$. Following Bell and Buneman, the maximum growth rate for the instability is[6]

$$\gamma_{\max} = 0.87\left\{\omega\left(\frac{\omega_s^2(\Omega_e+\omega)\,\omega\sin\theta}{4\omega_p{}^2\Omega_e(\Omega_e\,\omega\cos\theta+\omega(\Omega)_e+2\omega)\cos\theta}\right)^{1/3}\right\}_{\omega=\mathbf{k}.\mathbf{v}}, \tag{4}$$

where $\omega = \mathbf{k}.\mathbf{v}$ is the condition for resonance; ω_s, ω_p, Ω_e, Ω_i represent, respectively, the plasma frequencies of the relativistic and non-relativistic electrons, and gyrofrequencies of the electrons and ions; **k** is the propagation vector of the wave. Equation (4) shows that there is growth of Cerenkov radiation for wave propagation at a finite angle to the magnetic field. There is no growth for the angle $\theta = 0$ and 180° because for these angles there is no electric field in the direction of the wave propagation. In the case of the magnetosphere, there is not sufficient amplification of Cerenkov radiation for nearly parallel propagation because the region of amplification is very small. For quasars, however, the region of amplification can be as large as 10^{13} cm and, therefore, waves propagating nearly parallel can be efficiently amplified. The maximum growth rate for the Cerenkov radiation at frequency $\omega \approx \Omega_e$ is

$$\gamma_{\max} = 0.87\Omega_e\left(\frac{\omega_s{}^2}{16\omega_p{}^2}\frac{\sin^2\theta}{\cos\theta}\right)^{1/2} \tag{5}$$

If we represent the amplification factor by

$$\eta = \frac{1}{2\gamma_{\max}}\exp(2\gamma_{\max}R/V_g)$$

where V_g is the group velocity, which can be shown to be

$$\frac{2\sqrt{2}}{3}\frac{\Omega_e}{\omega_p}c(\cos\theta)^{1/2}$$

(from equation 3) and R is the region of amplification. For a typical optical luminosity of a quasar of 10^{46} erg s^{-1} (3C 273)[4], we obtain the relation $R^3 = 10^{37}\eta$. For an angle of propagation to the magnetic field < 5° and a typical value of $\gamma_{\max} = 4$, the amplification can be as high as 10^3 in the region $R = 10^{10}$ cm, which is much smaller than 10^{14}–10^{16} cm (refs 4, 5). It is therefore possible that the optical luminosity of quasars may be due to the amplification of Cerenkov radiation.

The amplification mechanism discussed here is based on the particles having free streaming along magnetic field lines. Such particles can be scattered, however, due to hydromagnetic waves as has been discussed in the case of the galactic cosmic rays[10]. As this scattering affects the amplification of the Cerenkov radiation, we must include an appropriate modification in our mechanism. Detailed calculations of the characteristics of Cerenkov emission and an estimate of its role in quasar luminosities are needed. It is especially important to use the Cerenkov amplification effect correctly to account for the high luminosities of quasars.

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Measuring solar wind velocity with spacecraft phase scintillations

THE discovery of interplanetary scintillations (IPS) of natural radio sources[1] has been followed by extensive multiple-station or spaced-receiver IPS measurements of the solar wind velocity in regions not yet probed by direct spacecraft[2-8]. Few measurements have, however, been obtained within 40 $R_\odot$ because amplitude or intensity scintillations saturate when they are strong as is the case near the Sun. This is unfortunate because the scarcity of wind velocity measurements in the acceleration region close to the Sun is one of the reasons why the construction of a unique picture of solar wind dynamics has not yet been possible, despite numerous theoretical studies and direct spacecraft observations farther out[9,10]. The demonstration of the measurement of spacecraft phase scintillations with a coherent dual-frequency radio system[11] paves the way for making solar wind velocity measurements with multiple-station phase scintillations. Although similar in principle to the IPS technique, several factors make this new tool a more powerful and promising one.

Because phase scintillations do not saturate when they are strong, observations can be carried out closer to the Sun than with amplitude scintillations. Spectral broadening has this same property[12] and we have obtained measurements at 2.3 GHz as close as 1.7 $R_\odot$ with the Helios spacecraft. Extensive phase scintillation observations in the accleration region of the solar wind are, therefore, possible using a dual-frequency S- and X-band coherent radio link[11].

For multiple-station observations the baselines are restricted to distances shorter than the correlation distance of the observed scintillations. For amplitude scintillations, the correlation distance is of the order of the size of the first Fresnel zone[13], which is roughly 140 km at 2.3 GHz. On the other hand, since the correlation distance for phase scintillations is at least 10^6 km (refs 11, 13), a much wider range of baselines is possible. Particularly suited for these measurements are the three 64-m S- and X-band tracking antennae of NASA's Deep Space Net located at Goldstone (California), Canberra and Madrid. These antennae can be used for two-station observations with baselines of the order of 10^4 km.

Because the slowly varying phase scintillations are considerably stronger than the faster varying amplitude scintillations[13], the signal–noise ratio (SNR) needed for long baseline phase measurements is much lower than that needed for short baseline amplitude measurements. The frequency spectrum of the phase scintillations[11,13] indiates that if the baseline distance is increased by a factor of 10 the SNR can be reduced by at least 20 dB. The choice of observing antennae for phase scintillations is, therefore, flexible, since a wide range of antenna sizes and receiver noise temperatures can be considered, in addition to the wide range of baseline distances. This is especially important since it would be desirable to add observing antennae to the group of NASA 64-m antennae to permit three-station measurements. Also, for a given SNR, slowly varying phase scintillations yield measurements at larger solar elongations than faster amplitude scintillations.

We have discussed some of the important advantages of measuring solar wind velocity with multiple-station phase scintillations. Current and future NASA deep space missions equipped with the coherent S/X radio system include Viking, Pioneer Venus and Mariner Jupiter/Saturn. Extensive observations using this new technique during the numerous superior conjunctions of these spacecraft will provide much needed velocity measurements in the acceleration region of the solar wind. These proposed observations will complement the large number of measurements that have been and are still being made with natural radio sources and will lead to a better understanding of the dynamics and driving mechanisms of the solar wind.

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Tidal acceleration of the moon deduced from observations of artificial satellites

DOUGLAS *et al.*[1] demonstrated the existence of an apparent latitude dependence of tidal friction by determining disparate values of the second degree Love number (k_2) from perturbations of the inclinations of the GEOS-1 and GEOS-2 satellites. Lambeck *et al.*[2] correctly explained this phenomenon as being due to neglect of ocean tide perturbations. Parameter values for some ocean tide components have been obtained from several satellites[3], but parameter values for the M_2 tide, the dominant (85%) effect of the oceans on the tidal acceleration of the Moon, have not been published. Using an improved method for computing mean elements, we[4] obtained an observation equation for the M_2 tide from the satellite 1967-92A. Applying this technique to the satellite GEOS-3, we now obtain an additional observation equation for the M_2 tide. As shown in ref. 2, solid and fluid tide effects on satellites cannot be separated, requiring assumption of the solid tide amplitude and phase parameters for a fluid tide solution. Assuming $k_2 = 0.30, \delta_2 = 0°$, and using the values of Lambeck[5] for the minor O_1 and N_2 contributions to n_t, our fluid tide parameters for the M_2 ocean tide yield the value of the tidal acceleration $n_t = -27.4 \pm 3$ arc s (100 yr)$^{-2}$, in excellent agreement with the value $n_t = -27.2 \pm 1.7$ arc s (100 yr)$^{-2}$ obtained by Muller[6] from a combination of ancient and modern observations. These two values are lower than the value $n_t = -35 \pm 4$ arc s (100 yr)$^{-2}$ obtained from numerical ocean tide models[5]. Our assumption of a negligible solid tide phase angle is supported by a recent determination by J. T. Kuo (personal communication) that the phase angle obtained from a transcontinental network of tidal gravimetric stations is < 1°. Changes $\leqslant 0.5°$ in solid tide phase angle change our result for the combined solid/fluid $\dot{n}_t$ by no more than 1 arc s per (100 yr) (ref. 2).

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Cosmic spherules as rounded bodies in space

COSMIC spherules obtained from deep-sea sediments have generally been accepted as extraterrestrial; but their mode of formation has been uncertain. On chemico-mineralogical evidence and the study of polished sections, it is difficult to refute the assumption that the spherules are merely the droplets shed from the fusion crust of a meteorite as it ablates in the atmosphere. We are now re-examining a new spherule collection (diameter > 50 μm) from surface red clay samples used previously[1]; that is, Atlantic A2 (24°30′ N, 64°47′ W, 5949-m depth) and Pacific (22°07′ S, 115°10′ W, 3,060–3,200-m depth). Scanning electron microscopy of 40 'stony' spherules and 25 'irons' has led us to consider a new hypothesis—that the cosmic spherule is a round body in space and those that we find suffer only slight alteration during their grazing flight through the atmosphere. Interesting surface features exist on many of the spherules, both stones and irons, which make it difficult to believe that they have been sprayed from a meteorite. If our hypothesis can be substantiated, then cosmic spherules become important objects in their own right, a cometary origin beingas distinct possibility. We now show some of these surface featur e and mention other supporting evidence. From the surface appearance we can classify the spherules into different types and forms, and all forms occur with similar relative frequency in Atlantic and Pacific oceans.

The 'stony' spherule type appears either as a vuggy form or an organised (near single crystal) form in about equal numbers. A vuggy form (Fig. 1), gives smooth lines of olivine and magnetite on X-ray powder photographs. The holes are suggestive of outgassing of some volatile substance during a warm stage. This example shows no sign of a fusion crust; under the optical microscope the surface is dark grey. We conclude (note the well-faceted crystals) that this spherule did not reach its melting point in atmospheric flight and was not attacked by seawater. From this and other examples, it seems that seawater has not affected our stones, collected from surface sediments. Stony spherules do erode, however; they are not found in the deeper sections of red clay cores.

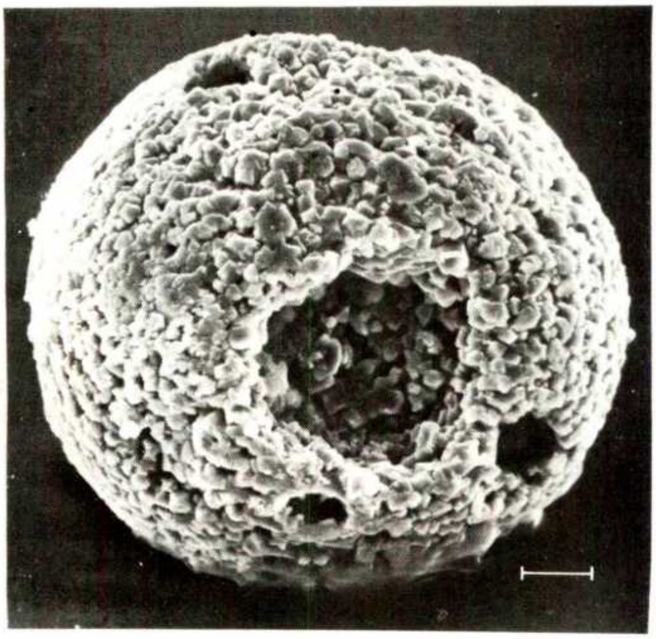

Fig. 1 A vuggy spherule. X-ray diffraction (all lines fairly smooth) gave magnetite lattice $a = 8.330$ Å, Fe/(Mg+Fe) = 22% for the atomic ratio of the olivine. On other vuggy spherules the crystal faces show interesting growth or etch features. Scale bar, 10 μm.

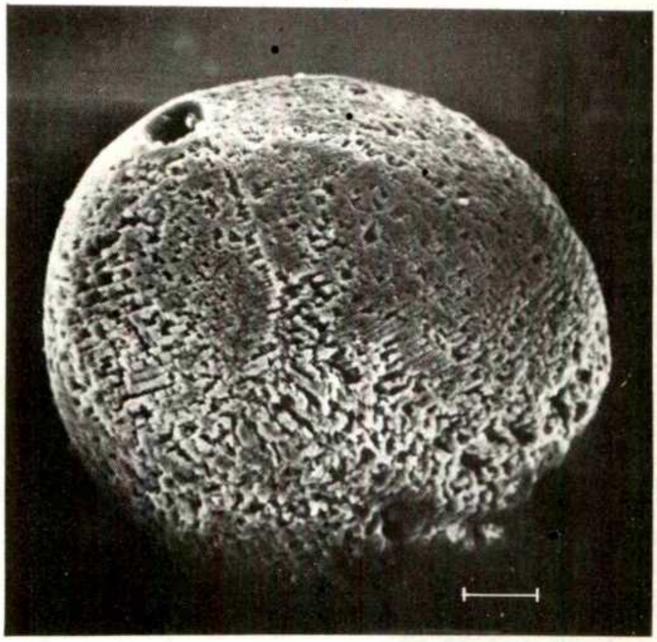

Fig. 2 An egg-shaped organised stone, uncommon because of the 'glassy' lined cupule found on the sharper pole. The spherule was polished to a diametral plane, including the cupule. The olivine crystals lie normal to the polar axis and no voids can be seen. Spot analyses of sub-micron areas gave: a somewhat variable Mg/Fe ratio; an erratic Ni distribution, which when present was ~0.1 Fe; and traces of Ca, Ti, Cr, Mn. X-ray diffraction (all lines very spotty) gave magnetite lattice $a = 8.356$ Å, and Fe/(Mg+Fe) = 20% for the olivine. From the spot analyses no large magnetite crystals were found in the interior. Part of the Ni seems to be in the olivine as well as in the magnetite. Ni in the stony phase of meteorites is < 300 p.p.m. (ref. 7). Scale bar, 20 μm.

Other vuggy spherules, sometimes found to be broken, show dark smoothish fusion crusts in patches. The interior is whitish and usually there is a large spherical hollow. In the unbroken state these vugs were thick-walled bubbles, with irregular-shaped holes scattered throughout the wall. X-ray microprobe analysis suggests that the magnetite is concentrated in the fusion crust. Fragile 'creamy-centred' spherules have already been described[1].

Fusion crusts on stony meteorites[2–4] are about 100 μm thick, vesicular and very opaque. In fact there is little or no glass present (private communication). Apparently magnetite is exsolved from the melt and the melt recrystallises into an olivine with a composition poorer in Fe than the parent material. For example, a pure olivine sample ablated in a plasma jet showed a 50% decrease of Fe[4]. In some respects both vugs and organised stones are similar to fusion crustal material. We find only olivine and magnetite (also sometimes wüstite). There is a lack of glass and the vugs are vesicular. However, the distribution of Fe/(Fe+Mg) for the olivine in our spherules agrees with that occurring in the interior of the chondrites[2]. If the spherules were ablation products, then the Fe content of the parent body would be greater than that commonly observed, a carbonaceous chondritic composition being more likely. But it is difficult, however, to see how vugs with whitish interiors could come from dark opaque material typical of a meteorite's crust. They seem more like micrometeorites, superficially fused or merely discoloured by moderate heating.

An organised form (Fig. 2) shows spotty olivine and magnetite X-ray lines. The texture is typical, but the presence of the 'glassy' lined cupule is very rare. In fact vesicles seem generally absent in the organised forms. The particle shown is slightly egg-shaped with the cupule near the sharp pole. We have found one stony spherule with a globule protruding about ⅓ of the way out of the surface. We mention this rare particle because it may provide the clue for explaining the cupule (Fig. 2). Perhaps during the molten stage, a magnetite globule was formed within the silicate melt and, assuming the melt was spinning, centrifugal forces would cause the globule to emerge at the sharper pole. Freezing may then flick the globule free. It could be argued that such a spinning process should occur at the moment of spraying from a meteorite's surface; and so egg-shapes, dumb-bells and even tear-drops would be a common occurrence. These forms are not found—all the spherules are near spheres.

Like the vugs, organised stones can be smooth in places. A thin fusion crust barely disguises the laminar or rod-like interior. The lack of a crust on the particle in Fig. 2 implies that it suffered little or no damage in its flight through the atmosphere. Again, seawater is unlikely to have removed a fusion crust, as the cupule is perfectly preserved. An SEM photograph in ref. 3 shows a 20-μm diameter spherule embedded in the fusion crust of Murchison. This is thought to have been sprayed off the surface of an 'upwind' piece of the shower and by chance to have been captured by the piece under investigation. This genuine ablation spherule shows a 'glassy' smooth surface. We have never found a stony cosmic spherule with such a smooth appearance, that is, having been thoroughly molten. The 'fusion' crusts on stony spherules seem very superficial. In some spherules parts of the crust have cracked away under thermal shock; in others the smoothing is only on one hemisphere and for these there is sometimes a 'brushed' appearance, strongly suggesting aligned flight through the atmosphere. Finally, the magnetite in the stones has a low lattice parameter, ranging around 8.352 Å; in the irons it ranges about a mean of 8.391 Å. Furthermore, we have never observed pyroxene in the spherules.

The 'iron' spherule type appears in two forms; those with a Ni-rich metal globule (or its oxidised remains) visible at the surface of a Ni-free magnetite–wüstite shell, and those without the metal—the oxide being only magnetite or magnetite–wüstite. A remarkable example of a metal form is shown in Fig. 3. It is the appearance of this particle that prompts us to view all our spherules as micrometeorites. It seems to us that in space the particle was already spherical, and flight through the atmosphere must have been steady, with the heavy globule forward. The frontal hemisphere is polished, as if a film of low melting point wüstite spread from the slightly dished taenite globule. Other particles show a spectrum of damage, ranging from metallic globules that are perfect, then dished (as in Fig. 3) to markedly cupped.

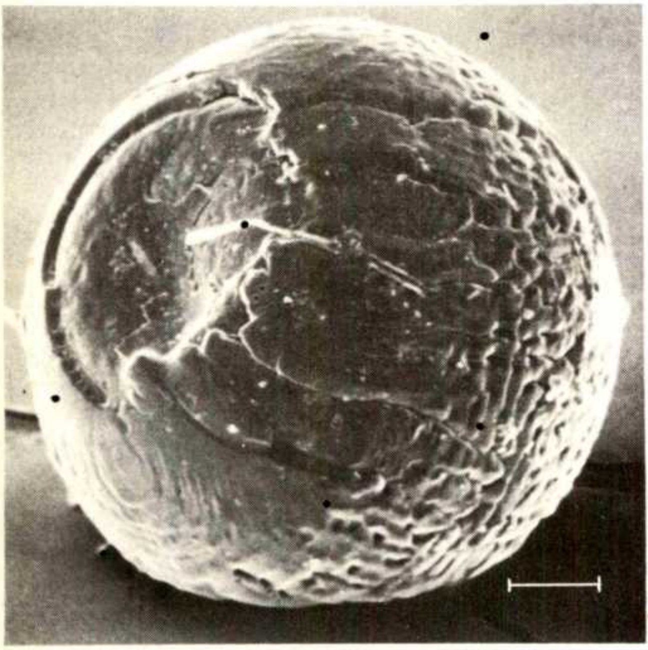

Fig. 3 A partly smooth ring structured iron—'Lucky Strike'. A polished section through the ring showed that the globule came right to the surface and had a diameter ~⅔ that of the whole. Note the shallow depression in the centre and the fractured edges of the ring. X-ray diffraction (all lines spotty) magnetite lattice a = 8.385 Å, wüstite lattice a = 4.286 Å, alloy lattice a = 3.573 Å suggesting 61% Ni. Scale bar, 10 μm.

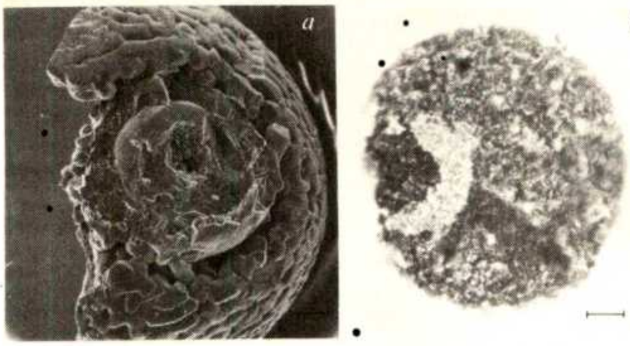

Fig. 4 A 'ballooned-iron' found as a hemisphere. *a*, the cracked-off outer part of the ballooned oxide and the small central void. Scale bar, 10 μm. *b*, An optical photograph of a polished section through the ring structure. Scale bar, 10 μm. A probe analysis for Ni gave ~1% in the magnetite shell, ~80% in metallic crescent and ~30% in the ballooned oxide. There is 4% Co in the metal, a trace in the shell but slightly more in the oxide scale. Spot analyses of sub-micrometre areas over the scale give Ni/Fe count ratios from 0.27 to 0.66, but many were constant at 0.55. This probably means that the oxide is nickel rich and contains in places some finely divided metallic nickel. X-ray diffraction gave magnetite (spotty lines) a = 8.390 Å, wüstite (spotty lines) a = 4.280 Å, alloy (smooth lines) a = 3.546 Å suggesting 82% Ni.

Other metal forms are more complex, and we call these 'ballooned-oxides'. Here a scale grows out from the globule as it oxidises. This expansion process can crack the spherule open, and a hemispherical portion is shown in Fig. 4*a*. In Fig. 4*b*, we show a polished section. All that remains of the globule is a crescent. The ballooned oxide scale within the arms of the crescent is rich in Ni, and there is little Ni in the oxide shell. This particle is similar to one found by Finkelman (Fig. 1 in ref. 5). Foley[6] reviewed the growth of non-molten scales on Ni/Fe alloys over a wide compositional range. For Ni ≳ 20% entrapment of Ni always occurs and a nickel spinel (trevoritic magnetite) forms. We now have to account for the lack of Ni in the oxide shell and the particle's general appearance. Clearly, at one time, the particle has been thoroughly molten and in this state Ni would diffuse to the metal phase. The particle then freezes. On entering the atmosphere at grazing incidence, the temperature does not reach the melting point (note the well-faceted crystals in Fig. 4*a*) but a non-molten oxide scale balloons out, thus cracking the spherule. Magnetite scales grow on metal under water, but this cannot be the case here. We have examples of ballooned forms clearly associated with melting conditions.

In the metal forms, the X-ray lines of the magnetite and wüstite phase are spotty, but the metal lines are sometimes smooth. In every case, the low lattice parameter (around 4.284 Å) for wüstite suggests that it has grown from magnetite rather than from the metal. Furthermore, the coincident positioning of the spots on the X-ray film show that wüstite and magnetite crystals are in near-parallel orientation.

The other iron form, lacking a noticeable metal phase, can give smooth X-ray lines. The surface can appear as a 'brick-work' of near-perfectly preserved crystals. In others, however, a thin film coats the crystals in places and the crystals can be large; the X-ray lines are spotty. Both the metal and non-metal forms can have very shiny surfaces, but it seems that this does not mean that the particle has been thoroughly molten. On soaking a shiny spherule in dilute nitric acid for a prolonged period, the metal globule apparently dissolved, and the shell broke into fragments on manipulation. Large and small crystals were seen in the shell material, but the shiny surface, which could not be resolved into crystals, is a distinct layer about 1 μm thick: a film which had spread roof-like across irregularities in the original surface. From the irons, we gain the impression that partial recrystallisation into larger crystals takes place during atmospheric heating, and if melting occurs it is only superficial.

We conclude that cosmic spherules seem to be most easily explained by assuming that they are already round in space. The spherules could come from the zodiacal cloud and those that we find have escaped vaporisation by entering the atmosphere at grazing incidence. Molten conditions must have existed at the time of their formation. Comets undergo violent eruptions and electric discharges may occur in their atmospheres. It is also possible that the refractory material within the comet is in droplet form as collisional heating could have occurred at the accumulation stage.

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Corrosion–fatigue crack initiation in metals

A DEFINITE relationship has been observed between the cyclic plastic strain-enhanced dissolution behaviour of metals in aqueous electrolytes and their corrosion–fatigue behaviour in similar conditions of environment and potential. The existence of such a relationship was first suggested in 1970[1], and the present work forms part of a wide study to examine the various effects of dynamic fatigue strains on electrode reactions at corroding metal surfaces.

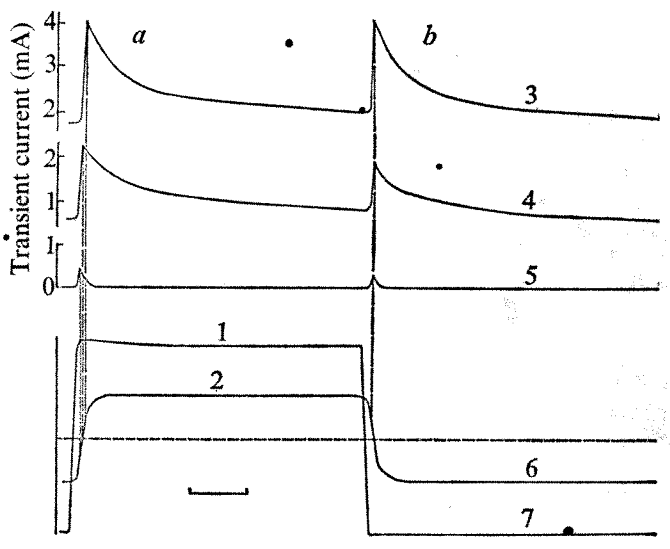

Fig. 1 Typical transients at the natural potential for mild steel in 3.5% NaCl solutions of varying *p*H. *a*, Tensile: 1, load −400 N mm^{-2}; 2, strain +0.03. *b*, Compressive: 3, *p*H 1; 4, *p*H 8.2; 5, *p*H 12; 6, strain −0.03; 7, load − 400 N mm^{-2}. Scale bar, 1 s.

The technique used involves strain cycling metal specimens under potentiostatic control in aqueous electrolytes and measuring the instantaneous electrode reaction kinetics using high-speed recording equipment. The apparatus and experimental procedure have been described in detail previously[2].

In the present tests a 'square' strain wave was used, giving strains of ±0.03 about zero at a strain rate of 0.03 s^{-1} and at a frequency of 0.1 Hz. Various metals were tested in different electrolytes over a wide potential range. Tests were carried out in conditions where corrosion–fatigue failure of the metal was known to occur and in conditions where this type of failure was not observed, so that the dissolution behaviour of the metal in 'failing' and 'non-failing' situations could be compared.

Mild steel suffers from corrosion–fatigue in aqueous sodium chloride solutions of *p*H 1–11 but is immune to corrosion–fatigue at *p*H 12. Figure 1 shows *U*–*V* recordings of the dissolution

Table 1 Corrosion–fatigue test and cyclic strain dissolution data for various systems

		Corrosion–fatigue test data		Cyclic strain dissolution data				
Material	Air fatigue limit (σ_a) (MN m^{-2})	Corrosion–fatigue endurance (σ_c) (MN m^{-2})	Test conditions material, test solution and potential		I_{peak} Values, First cycle (mA)	(±3% strain) 15th cycle (mA)	Sign of $\frac{d\Delta I}{dN}$	$\frac{\text{Cor. fat. end. limit}}{\text{air fat. end limit}}\left(\frac{\sigma_c}{\sigma_a}\right)$
0.18% C steel	250	55 (4) (freely corroding in 3% NaCl of neutral *p*H)	0.2% C steel in 3.5% NaCl solution *p*H 8.2 (at the natural potential)	Tensile	0.9	1.85	+ve	0.22
				Compressive	0.8	1.15		
Al–Zn–Mg alloy	138	69 (5) (freely corroding in 3% NaCl of neutral *p*H)	Al–Zn–Mg in 3% NaCl solution *p*H 6.5 (at the natural potential)	Tensile	70μA	111 μA (±1% strain)	+ve	0.5
				Compressive	3.5μA	79 μA		
0.18% C steel	275	275 (6) (freely corroding in 3% NaCl *p*H 12)	0.2% C steel in 3.5% NaCl solution *p*H 12 (at the natural potential)	Tensile	1.25	0.4	−ve	1.0
				Compressive	1.0	0.2		
0.19% C steel	185	194 (7) (in 10% NH_4NO_3 sol. at 0.6 V (s.c.e.)	0.2% C steel in 10% NH_4NO_3 (at 0.6 V (s.c.e.))	Tensile	1.0	0.4	−ve	1.05
				Compressive	0.5	0.25		
0.42% C steel	270	270 (8) (freely corroding in 3% NaCl solution +0.05 M Na_2CrO_4)	0.2% C steel in 3.5% NaCl solution + 0.05 M Na_2CrO_4 *p*H 8.2 (at the natural potential)	Tensile	0.7	0.4	−ve	1.0
				Compressive	0.6	0.3		
Titanium	466	466 (9) (freely corroding in 3% NaCl solution of neutral *p*H)	Titanium in 3.5% NaCl solution *p*H 8.2 at the natural potential)	Tensile	2.0	0.2	−ve	1.0
				Compressive	0.6	0.1		
Monel	265	265 (4) (freely corroding in 3% NaCl solution of neutral *p*H)	Monel in 3.5% NaCl solution *p*H 8.2 (at the natural potential)	Tensile	0.5	0.2	−ve	1.0
				Compressive	0.2	0.12		

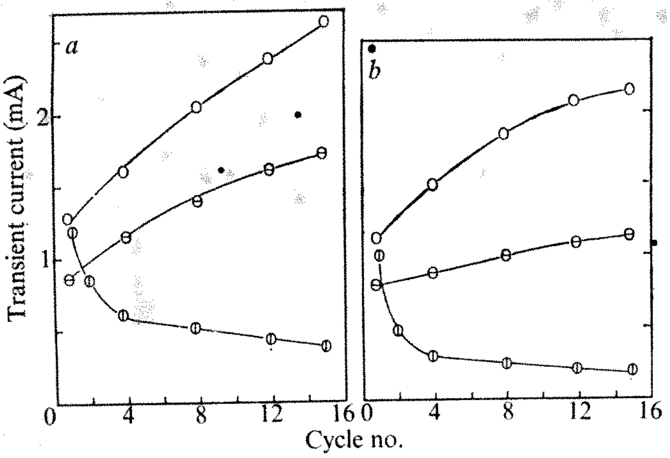

Fig. 2 The variation in the transient peak value with strain cycle number. *a*, Tensile: ○, *p*H 1; ⊖, *p*H 8.2; ⦶, *p*H 12.

transients produced by strain cycling mild steel in 3.5% NaCl solution of *p*H 1.0, 8.2 and 12.0 at the appropriate natural potentials. The transients relate to the fifteenth strain cycle. Figure 2 shows how the transient current magnitude, ΔI (defined as $I_{\mathrm{maximum}} - I_{\mathrm{initial}}$), varied with cycle number, N. ΔI, for the first strain cycle, was similar in all cases, but at *p*H 1.0 and 8.2 it increased with subsequent strain reversals whereas at *p*H 12 ΔI decreased progressively. Hence for the corrosion–fatigue failure situation $\mathrm{d}\Delta I/\mathrm{d}N$ was observed to be positive whereas for corrosion–fatigue immunity $\mathrm{d}\Delta I/\mathrm{d}N$ was negative. Other systems gave transients with similar characteristics, and the results are compared in Table 1 with the known corrosion–fatigue behaviour in similar conditions.

In all cases $\mathrm{d}\Delta I/\mathrm{d}N$ is positive when corrosion reduces fatigue life, that is when the ratio

$$\frac{\text{corrosion–fatigue endurance limit}}{\text{air fatigue endurance limit}}$$

is significantly less than one.

When the ratio equals one, $\mathrm{d}\Delta I/\mathrm{d}N$ is always negative. No exceptions to this rule have been found in the systems so far investigated.

The number of fatigue cycles to failure of stainless steel in sulphuric acid at different potentials was previously shown to be related to the magnitude of cyclic-strain-enhanced-dissolution effects[3]. It is now apparent that this principle can be successfully extended to explain the susceptibility and immunity of metals to failure by corrosion–fatigue.

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Auger electron spectroscopy of wear surfaces

THE effectiveness of lubricants containing sulphur and/or chlorine compounds in preventing wear and seizure of steels is related to the chemical lability of the load-carrying element (sulphur, chlorine) contained in them[1]. This finding is consistent with the formation of surface layers of iron sulphide and chloride which prevent metal wear and seizure. Little could be deduced, however, about the nature of these protective layers. We investigate here the surface region of wear scars formed in the four-ball lubricant testing machine using Auger electron spectroscopy (AES) incorporating an argon ion sputtering technique. Here a beam of argon ions is used to etch away surface layers, and expose underlying layers for examination by AES. Using these techniques we can build up an approximate composition profile for the surface region of the sample. This was a more detailed investigation than that by Buckely[2], who used AES principally to investigate changes in the surfaces of loaded disks.

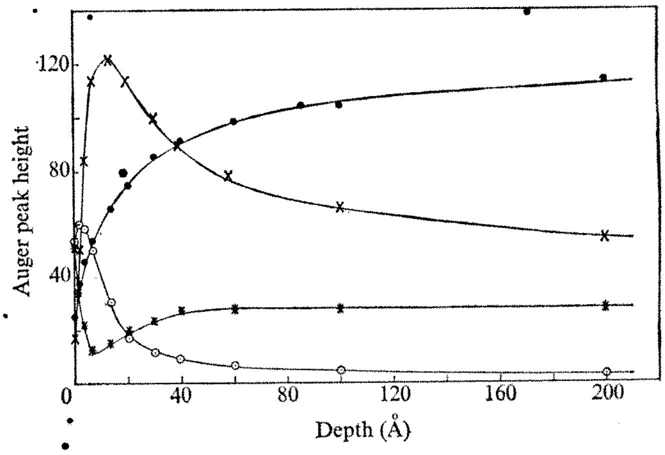

Fig. 1 Wear scar profile using dibenzyl disulphide. ●, Fe; ×, s; *, c; ⊙ O. The abscissa displays a depth scale in Ångstroms constructed on the basis of an ion beam current density of 10^{-2} (1 μA cm^{-2}) producing a sputtering rate of 1 Å min^{-1}. This conversion is arbitrary, and takes no account of differential sputtering rates of various materials. Initial results of sputtering rates determined for pure metals has shown it to be of the right order, though somewhat too conservative.

AES was carried out using a Varian cylindrical mirror analyser incorporating a 10-kV electron gun equipped with a scanning

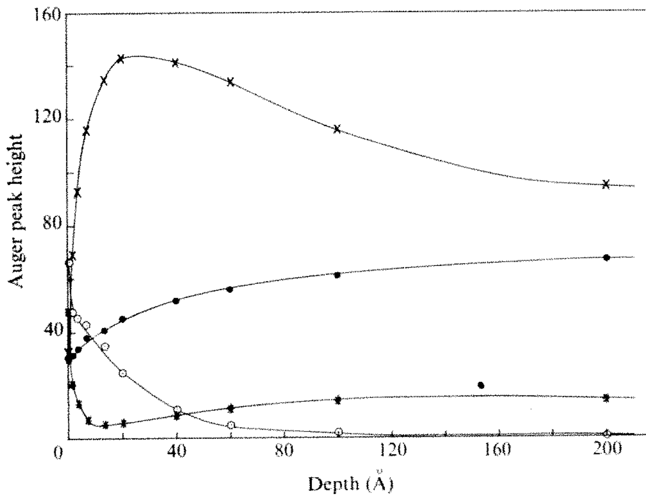

Fig. 2 Wear scar profile using di-*tert*-nonyl polysulphide. Symbols as in Fig. 1.

control for examination of areas from a few tenths of a square millimeter down to a focused spot of 2×10^{-5} mm^2. We used a 5-kV beam of 10^{-6} Å over an area of 0.1 mm^2. A large area was examined to minimise effects due to surface inhomogeneity, and the low electron current density (10 A m^{-2}) reduced beam effects. A Varian 3-kV argon ion gun in a pressure of 8×10^{-3} N m^{-2} (6×10^{-5} torr) of argon was used for argon ion sputtering. The ion beam was scanned across the surface to ensure an even ion current density which was measured using a Faraday cup. Sputtering was carried out to at least 2,000-Å depth but the most important changes occurred in the first few hundred Ångstroms, and this range is shown in the profiles (Figs 1–5).

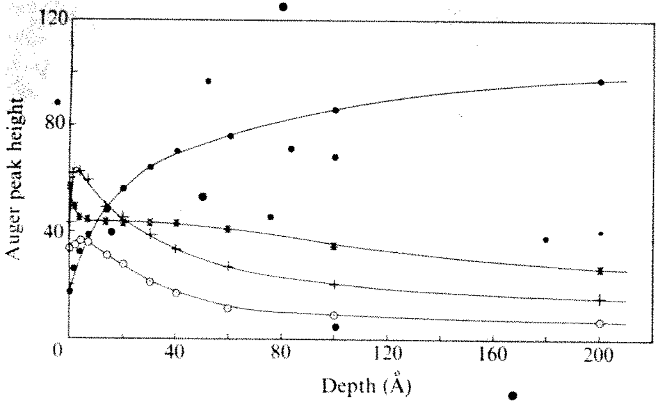

Fig. 3 Wear scar profile using Cereclor 51L. Symbols as in Fig. 1. + Cl.

We examined two sulphur-containing compounds, dibenzyl disulphide and di-*tert*-nonyl polysulphide, and two chlorine-containing compounds, Cereclor 51L (a commercial chlorinated hydrocarbon) and benzyl chloride, as lubricant additives. Blends were made up containing 15 mg-atoms of either sulphur or chlorine in 100 g of light liquid paraffin, and tested at a load of 160 kg for 1 min. The wear scars produced were washed twice in Analar toluene before examination in the Auger electron spectrometer.

The profiles obtained using the sulphur-containing oils (Figs 1 and 2) show that both oils afforded wear scars showing a high level of sulphur, presumably as iron sulphide, in the surface region. The sulphur level fell away very gradually with increasing depth, indicating considerable reaction with the metal over the first few hundred Ångstroms. High levels of carbon and oxygen were present at the surface, but were greatly reduced on etching, and can probably be ascribed to atmospheric contamination.

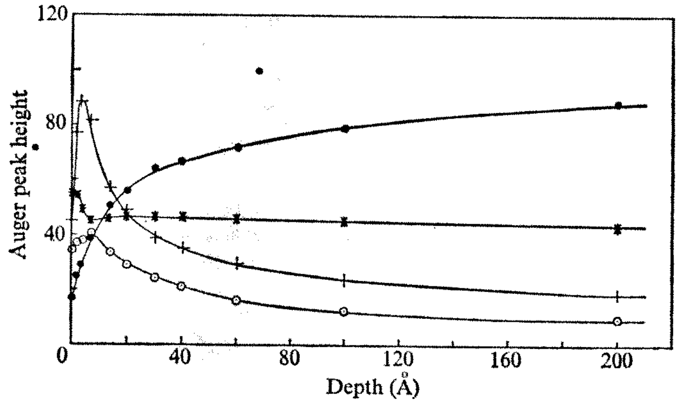

Fig. 4 Wear scar profile using benzyl chloride. Symbols as in Figs 1 and 3.

When the scars from the balls lubricated with chlorine-containing additives were examined (Figs 3 and 4), both additives showed a high chlorine level at the surface, but the level fell away sharply on etching, suggesting that only a very thin layer of iron chloride was formed. Also, a high, persistent level of carbon was found, and this was generally greater than the carbon level found with sulphur-containing oils and remaining after etching.

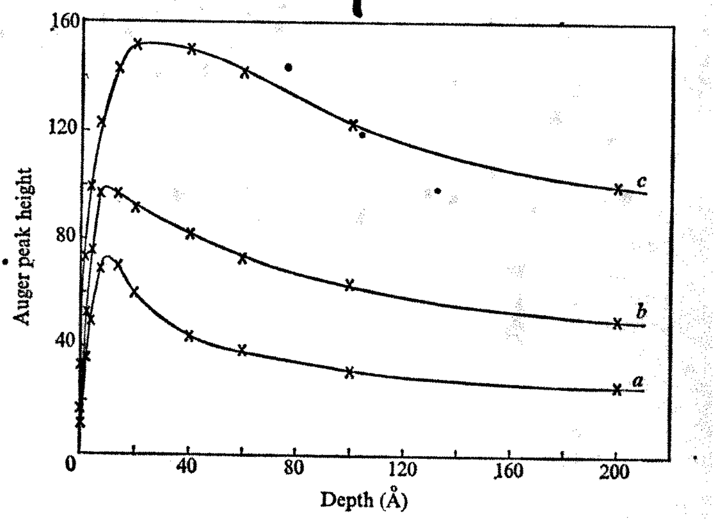

Fig. 5 Sulphur Auger profiles using di-*tert*-nonyl polysulphide at various loads. *a*, 50 kg; *b*, 100 kg; *c*, 160 kg.

Certain correlations are apparent between the wear scar profiles obtained in this work and the lubricating properties of the additives. In high load conditions, the sulphur-containing oils perform better than the chlorine-containing oils as shown by the wear scar diameters: dibenzyl disulphide, 1.54 mm; di-*tert*-nonyl polysulphide, 1.48 mm; Cereclor 51L, 2.39 mm; benzyl chloride, 2.18 mm. This effect could be linked to the production of thicker layers of iron sulphide compared with iron chloride. Furthermore, comparison of the two sulphur-containing oils revealed that di-*tert*-nonyl polysulphide gives a higher maximum concentration and deeper penetration of sulphur, and is a better extreme pressure additive than dibenzyl disulphide. The improved performance of benzyl chloride compared to Cereclor 51L is consistent with the higher chlorine level at the surface produced by the more reactive compound.

As the pressure on the bearing surfaces is one of the most critical of the parameters affecting wear, we have also examined the effect of load on film formation. The sulphur profiles (normalised to the same final iron level) obtained from wear scars formed at different loads using a blend of di-*tert*-nonyl polysulphide in liquid paraffin (Fig. 5) show that, over the range examined, both the maximum sulphur concentration and the degree of sulphur penetration increases with load. The increase in the sulphur level near the surface with increasing load is obviously related to the particularly good lubricationg properties of sulphur-containing oils in extreme conditions.

Examination of scars produced using mixtures of Cereclor 51L and the two sulphur compounds in liquid paraffin produced profiles showing the essential features of those obtained from the individual additives. A fuller account of this work will be published shortly.

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Structural imperfection and morphology of crocidolite (blue asbestos)

ELECTRON microscopic studies of fibrous materials, including a range of chain silicates[1–6], synthetic polymers[7] and carbon fibres[8], have almost exclusively involved examination of the structural characteristics visible when the fibre axis is perpendicular to the electron beam. It is extremely difficult to prepare ultra-thin sections of fibrous samples such that this axis is parallel to the beam; and yet it is in this projection that the greatest insights into structural and morphological irregularities are likely to be achieved, a fact well illustrated by the high-resolution studies of Yada[9] on chrysotile asbestos which, being less brittle than crocidolite and other amphiboles, was amenable to the normal techniques of ultra-microtomy. We present here preliminary results of a successful axial study of crocidolite (blue asbestos), the fibrous form of the amphibole mineral riebeckite (idealised formula $Na_2Fe_5(Si_4O_{11})_2(OH)_2$, space group C2/*m*, $a = 0.973$, $b = 1.806$, $c = 0.533$ nm, $\beta = 103.3°$). The electron micrographs reveal several novel features.

Crocidolite from Wittenoon, W. Australia, was embedded by vacuum impregnation with Araldite (CY212) resin and thin sections (~ 2 mm) were cut across the fibre axis using a diamond saw. These sections were mechanically polished to a thickness of ~ 50 μm and finally thinned by argon-ion bombardment at a glancing angle of 12°. They were examined in Philips EM300 and Siemens Elmiskiop 102 electron microscopes using a double tilt stage that enabled the fibre axis to be set exactly parallel to the electron beam. With this orientation both x^* and y^* are normal to the electron beam so that the $(hk0)$ reflections generate lattice fringes which serve as means of identifying the precise disposition of each constituent fibril with respect to its neighbour and, in addition, of characterising planar and other structural faults.

Figure 1*a* shows a typical section, and in its schematised analogue (Fig. 1*b*) the profiles of the fibrils have been delineated and their relative degrees of rotation around the *z* axis indicated. The electron transparent regions separating the fibrils demarcate the Araldite cement, which before hardening had penetrated the fibre and separated the fibrils slightly. The overall irregularity of the fibril profiles, notwithstanding some possible artefacts due to the ion-beam thinning, is surprising, as only occasionally do the fibrillar faces exhibit crystallographic indices which coincide with the low-index cleavage faces (010) and (110), seen in optical studies[10].

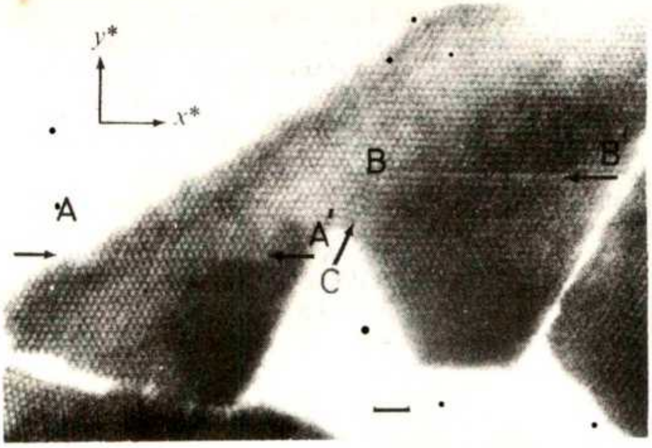
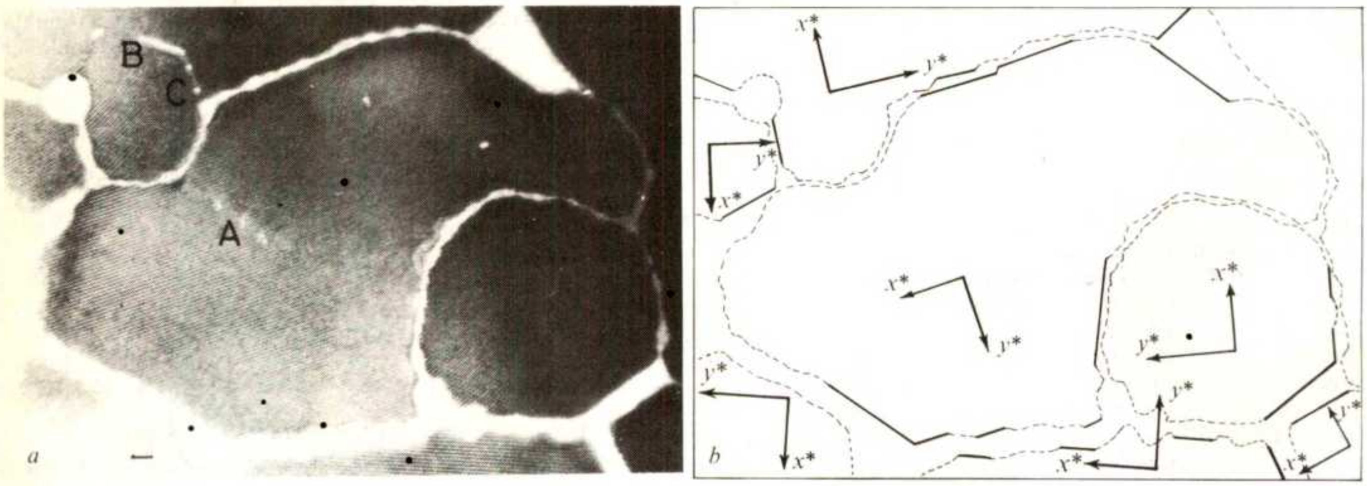


Fig. 2 Lattice image, along fibril axis, of crocidolite showing planar faults at AA′, BB′ (which coincide with (020) planes) and BC, which is the trace of (110). AA′ and BB′ are Wadsley defects consisting of triple-chains (see text). Scale bar, 5 nm.

The electron-transparent regions located within the fibrils (A, B . . . in Fig. 1*a*) arise from isolated but aligned grown-in defects, which could well have been preferentially etched during ion bombardment. The possibility that they are micropores, incorporated during growth, cannot be discounted: the dimensions of the pores range from 2 nm (that is, corresponding to a unit-cell wide 'pipe') to over 10 nm in diameter. The alignments of these features could be the result of small-angle boundaries, for example, the (010) family may be interpreted either in terms of a tilt boundary composed of dislocations of the type (100)[010] or in terms of dislocations lying on (010) slip planes with Burgers vector in the direction [100].

So far as the planar faults are concerned (Fig. 2), those denoted AA′ and BB′ lie parallel to (020) and, by analogy with previous studies[3,5], it is almost certain that these correspond to triple-chain lamellae that have been incorporated into the parent double-chain matrix. They are examples of Wadsley defects[3,5] and their presence in crocidolite is demonstrated here for the first time. At such faults there is departure from the normal chemical composition of the mineral. At the triple-chain regions a reduction in number of O atoms bonded to Si is compensated by an equal increase in the number of OH

Fig. 1 *a*, High resolution lattice image of ion-thinned crocidolite with the electron beam parallel to the fibrillar (*z*) axis. *b*, Schematised form of *a*. The reciprocal lattice directions are indicated, and the random orientation of the fibrils about their axes is apparent. ——, Fibril boundaries corresponding to low-index planes. The fibrillar outlines (— — —) show a general irregularity.



groups. Planar faults on (110) are also present (BC, Fig. 2), the displacement vector of this extended defect, as measured on the micrograph, being b/4 [010]. It is noticeable that planar faults CB and BB′ intersect, and the nature of the structural rearrangement that occurs in the vicinity of region B is not yet clear.

The precise significance and full characterisation of planar faults in crocidolite (as well as in other related brittle minerals which may now be studied by the technique herein described) awaits the results of dynamical calculation of lattice images[10,11]. Such calculations are under way.

We thank the SRC and the Royal Society Exchange Scheme for support that enabled M.A.F. to work in these laboratories. We are indebted to Dr E. J. W. Whittaker for drawing our attention to this problem, and for his helpful comments on the micrographs. The technical assistance of Mr. J. L. Jenkins is also appreciated.

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Pyrolysis of a polyborodiphenylsiloxane

WE have previously reported successful production of a high-strength continuous SiC fibre[1–3], a high flexural strength SiC sintered body[4] and a heat-resistant Fe–Cr alloy[5] by converting organometallic polymer polycarbosilane (PC) as the binder to an inorganic in thermal decomposition. We then found that in the thermal decomposition of organometallic polymers such as PC, an amorphous state exists before formation of the (SiC) fine particles; this SiC amorphous state is hardly possible in inorganic reactions. The process of thermal decomposition of organometallic polymers is little known. To obtain general information on the phenomenon, we have newly synthesised multi-element polyborodiphenylsiloxane containing oxygen (B, C, Si, O, H) compared with PC composed of Si, C and H, and observed the process of its thermal decomposition.

Polyborodiphenylsiloxane, (PB)p was synthesised as follows. Boric acid (0.33 mol, 20.6 g) and diphenyldichlorosilane (0.5 mol, 126.6 g) were dissolved in 200 ml of anhydrous *N*-butyl ether. The solution was then refluxed in N_2 gas atmosphere for 18 h, and heated for 1 h under reduced pressure at 300 °C, successively. 102 g of (PB)p was obtained as 92% yield. The (PB)p synthesis display is represented by the following reaction

$$3[(Ph)_2SiCl_2] + 2H_3BO_3 \rightarrow [(C_6H_5)_6Si_3B_2O_6] + 6HCl\uparrow$$

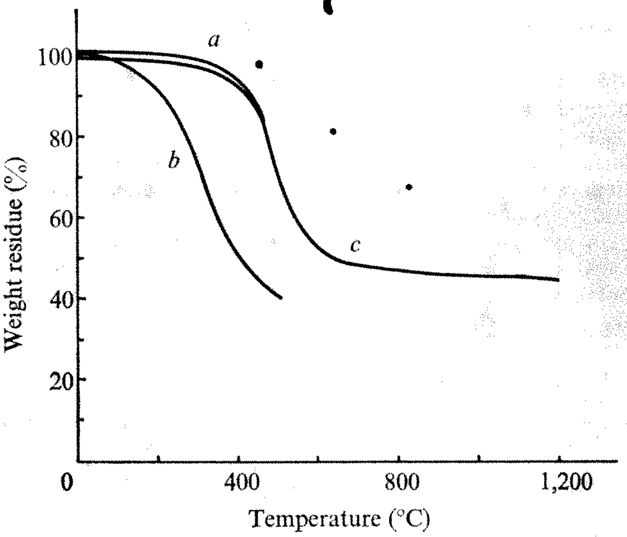
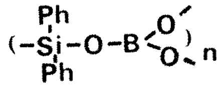


Fig. 1 Thermal analysis curves of (PB)p and (PB)m. *a* and *b*, TG curves of (PB)p and (PB)m respectively, up to 500 °C from room temperature in air. TG curve of (PB)p up to 1200 °C from room temperature in a stream of N_2 gas.

The intermediate product, $[(C_6H_5)_6Si_3B_2O_6]$, is pyrolysed at 300 °C. Microchemical analysis values of the product (PB)p are shown in Table 1, with the expected theoretical ones as $[(C_6H_5)_6Si_3B_2O_6]$. The observed analytical values of the (PB)p, C and O contents are reduced in comparison to the theoretical ones and the difference can be attributed to the pyrolysing process at 300 °C of the $[(C_6H_5)_6Si_3B_2O_6]$. The softening point is 100 °C. Its infrared spectrum shows peaks at 3600 cm^{-1} (free OH), 3500–3100 cm^{-1} (hydrogen-bonded OH), 1600 cm^{-1} (C_6H_5), 1500–1300 cm^{-1} (B–O) and 1150–1000 cm^{-1} (Si–O), respectively. The product was found to be (PB)p. It is considered to contain partial structures as follows



$$\left(-\underset{\text{Ph}}{\overset{\text{Ph}}{\text{Si}}}-\text{O}-\text{B}\begin{matrix}\text{O}\\ \text{O}\end{matrix}\right)_n$$

Of the various organometallic polymers, boron polymers are generally heat resistant but not very moisture proof[6]. Vale reported the producton of polyborodimethylsiloxane (PB)m from boric acid and dimethyldichlorosilane[7]. He improved its moisture-proof property by special means of electron irradiation. The (PB)p synthesised was found to be far more moisture proof than (PB)m. When both (PB)p and (PB)m were left in air at room temperature for 1 week, the transparence of the (PB)p was not changed, but the (PB)m was appreciably devitrified.

Simply by using diphenyldichlorosilane instead of dimethyldichlorosilane, we obtained here a superior moisture-proof characteristic of (PB)p facilitating practical handling. The thermal decomposition process of (PB)p and (PB)m was compared by means of a thermobalance. Figure 1 shows thermal

Table 1 Composition (wt%) of polyborodiphenylsiloxane, (PB)p heated 1,000 °C and (PB)p heated 1,500 °C

Sample	B	Si	C	O	H	N
(PB)p: experimental	3.68	16.23	59.1	7.8	4.9	< 0.2
theoretical*	3.26	12.68	65.06	14.45	4.55	—
Heated (1,000 °C) (PB)p	5.46	37.52	(~ 44)	12.2	0.5	< 0.2
Heated (1,500 °C) (PB)p	5.08	44.8	35.6	12.2	0.7	1.5

*The theoretical value is calculated as $[(C_6H_5)_6Si_3B_2O_6]$.

analysis curves of (PB)p and (PB)m from room temperature to 500 °C in air. TG analysis of (PB)p was examined up to 1,200 °C, in a stream of N_2 gas, and the TG curve was almost same as that of air up to 500 °C. The thermal decomposition initiation temperature of (PB)p is about 150 °C higher than that of (PB)m, indicating its superior heat resistance, and the weight loss is also smaller. (PB)p was thus greatly improved in both heat resistance and in its moisture-proof characteristic.

In pyrolysed (PB)p, the organic group is decomposed. At 1,000 °C, an amorphous phase consisting of many elements is obtained (Fig. 2*b*). Analytical values of the pyrolysed product at 1,000 °C, and 1,500 °C in N_2 gas atmosphere are shown in Table 1; and the residual as decomposition product at 1,000 °C is about 46%. The amounts of SiC of 1,500 °C sample should be at least 37 at%, based on the following assumption: all of oxygen atoms react with silicon atom and the remaining silicon atoms react with carbon and convert to silicon carbide. By further pyrolysis of the product up to 1,700 °C in Ar gas flow, phases of abundant β-SiC, glassy carbon and slight B_4C appear (Fig. 2*c*). In Fig. 2*c*, diffraction peaks (arrow) were few and weak, but they coincided with ASTM card, 6–0555 (B_4C) and the infrared spectrum of 1,700 °C (PB)p was coincided with that of a B_4C compound. The crystalline product due to the peaks is considered to be a B_4C compound.

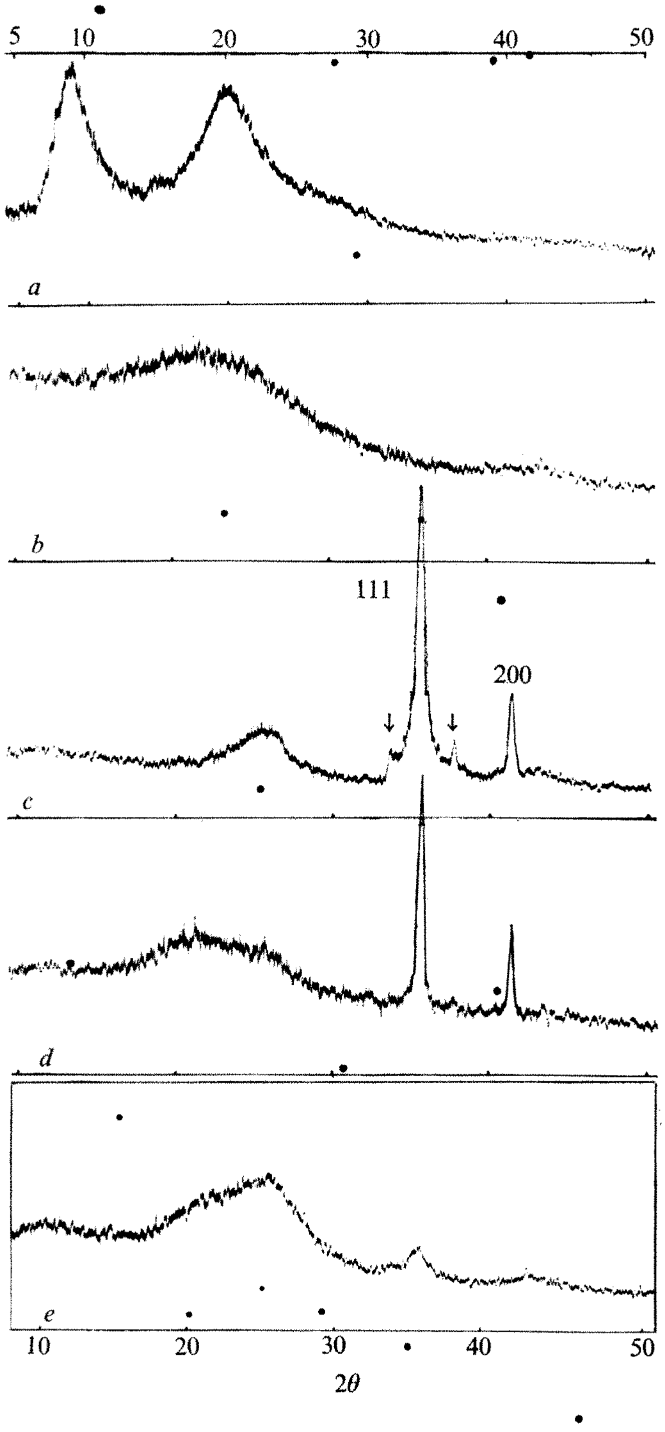


Fig. 2 X-ray diffraction patterns (CuKα) of (PB)p and the pyrolysed (PB)p products at respective temperatures. *a*, Polyborodiphenylsiloxane; *b*, 1,000 °C heat-treated (PB)p in a stream of N_2 gas; *c*, 1,000 °C product heated at 1,700 °C in Ar gas for 1 h; *d*, 1,700 °C product heated at 1,400 °C in air for 2 h; *e*, 1,000 °C product heated at 1,400 °C in air for 2 h. '111, 200': diffraction peak in β-SiC.

Though constituents of (PB)p are many (including Si, B, C and O), on pyrolysis in a reducing atmosphere, its products contain mostly a β-SiC product. This phenomenon is important.

The 1,700 °C product was then burnt at 1,400 °C in air. In the X-ray diffraction, in addition to the β-SiC pattern, broader pattern than that at 1,700 °C appears between 2θ = 16° and 30° (Fig. 2*d*). The compound, B_4C is considered to be composed and converted to amorphous B_2O_3. The broad pattern is probably ascribed to the glassy phase of B_2O_3 and the amorphous (PB)p residue and glassy carbon.

On the other hand, when the product at 1,000 °C is further burned at 1,400 °C in air, amorphous-like SiC and an amorphous phase of multiple elements occurs (Fig. 2*e*).

Because pyrolysis at up to 1,700 °C produces SiC, B_4C, and so on, it is easy to convert (PB)p into a heat-resisting inorganic material. There is thus a possibility of using (PB)p as a binder in the SiC body, for example; this will be described in another report.

In conclusion, in both previously developed polycarbosilane and newly-developed polyborodiphenylsiloxane of multiple elements, there is an amorphous state during thermal decomposition to such heat-resisting inorganic compounds as SiC and B_4C. Therefore, the production of an amorphous state in the thermal decomposition of organometallic polymers to inorganics seems to be common.

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SiC and Si_3N_4 sintered bodies with new borodiphenylsiloxane polymers as binder

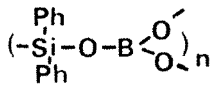
HIGH-STRENGTH SiC and Si_3N_4 sintered bodies have been studied for their application as high-temperature structural materials[1]. A high-strength SiC sintered body using an organometallic polymer as the binder has been reported[2]. A feature of this method is that polycarbosilane (PC) as the binder is converted into fine-particle inorganic β-SiC by heat-treatment, which fills the grain boundaries of the matrix SiC. In this way, with a hard-to-sinter material such as SiC or Si_3N_4, a sintered body with excellent mechanical strength can be produced at relatively low temperature

Table 1 Sintering conditions and properties of the sintered bodies

Matrix	Step	Conditions	Apparent density (g cm^{-3})	Flexural strength (MN m^{-2})	Fig.
SiC	a	Heating at 100 °C h^{-1}, keeping at 1000 °C for 1 h; N_2 gas flow	2.55	80	*a*
	b	Rapid heating, keeping at 1700 °C for 1 h; 1.8 MPa Ar	2.43	109	*b*
	c	Rapid heating, keeping at 1400 °C for 40 h; in air	2.49	182	*c*
Si_3N_4	a	Heating at 100 °C h^{-1}, keeping at 1000 °C for 1 h; N_2 gas flow	2.13	62	
	b	Rapid heating, keeping at 1500 °C for 1 h; 1.8 MPa Ar	2.06	71	
	c	Rapid heating, keeping at 1000 °C for 40 h; in air	2.18	125	

without using a hot press. We recently synthesised a particular form of polyborodiphenylsiloxane (PB)p, an organometallic polymer, which is very effective as a binder. Using this binder, SiC and Si_3N_4 sintered bodies of high mechanical strengths could be successfully obtained. These results are outlined here.

A partial structure of (PB)p used as the binder is shown as



The method of synthesis properties of (PB)p and phenomena in pyrolysing (PB)p are described elsewhere[3]. The purity and mean particle size of both the α-SiC and the β-Si_3N_4 used as the matrix materials were 99.9% and 30 μm, respectively. The procedure giving the best result in a sintered body is as follows. About 10% by weight of (PB)p is added to the SiC or Si_3N_4 powder. To this mixture, benzene is then added, to dissolve the (PB)p. The result is a uniform mixing of binder and matrix powder. The solvent is evaporated off, and a green body of size $10 \times 30 \times 4$ mm^3 is prepared by mould-pressing at 196 MPa. The subsequent sintering process, in three steps, is shown in Table 1.

The flexural strength of the sintered SiC and Si_3N_4 bodies at room temperature was measured by the method previously described[2]. The measured results and the densities are shown in Table 1. Scanning photomicrographs of the fractures after the successive steps of sintering are given in Fig. 1.

From X-ray diffraction after the successive steps of sintering of a SiC body (Table 1, Fig. 1), the following conclusions emerged. In step (a), the amorphous binder, not yet well crystallised surrounds the SiC particles. In step (b), the (PB)p binder has now been mostly converted into fine-particle β-SiC. Between 1,000 and 1,700 °C, flow of the amorphous binder would occur, resulting in good wetting of the matrix α-SiC. The flexural strength of the sintered body thus becomes larger than in step (a). X-ray diffraction at this stage showed Bragg peaks due to small amount of B_4C and amorphous carbon, as well as β-SiC. In step (c), the B_4C peaks disappear and the diffraction intensity indicating an amorphous phase increases. B_4C has thus become a glassy material by reaction with oxygen in the air. This glassy substance acts as a binder for the matrix SiC powder. Possibly because of this, the flexural strength of the sintered body is further raised. Similar results were obtained with the Si_3N_4 sintered body. Therefore, the sintered SiC and Si_3N_4 bodies using (PB)p as the binder will be safely usable at high temperatures and even in an oxidising atmosphere.

As seen in Table 1, apparent densities of the sintered bodies are relatively low. By impregnation of the sintered body with (PB)p, followed by further heat-treatment, therefore, the apparent density and the flexural strength could both be further increased. The method is similar to that described elsewhere[2]. By applying this process to the SiC and the Si_3N_4 sintered bodies following step (a), the increases in density and strength per impregnation were about 0.09 g cm^{-3} and 60 MN m^{-2}, respectively, in each case.

Compared with PC, (PB)p can be more easily synthesised and is more effective as the binder for obtaining high-strength sintered bodies.

In the SiC and Si_3N_4 sintered bodies using (PB)p as the binder, there is no dimensional change on sintering. The method of converting (PB)p to an inorganic as the binder is useful, and original, in industrial production. The sintered bodies, which are of high strength, should find many applications in various fields.

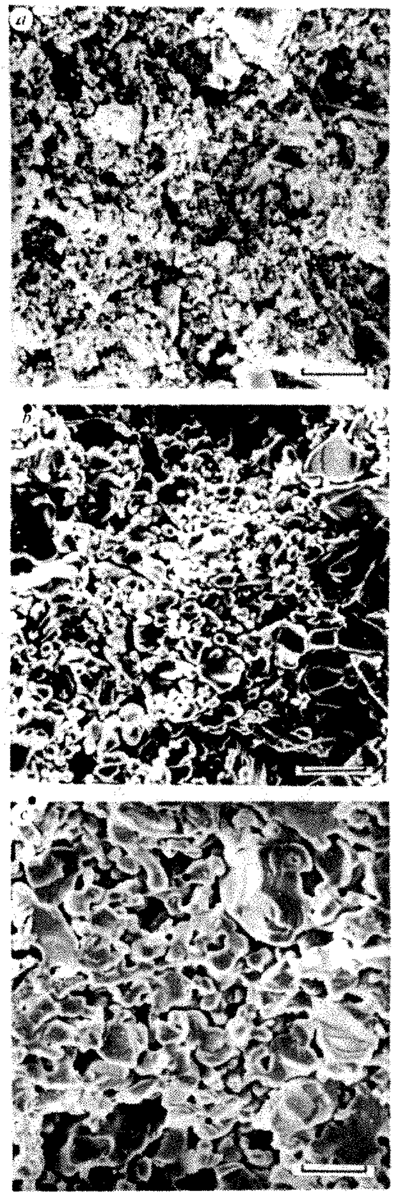


Fig. 1 Scanning photomicrographs of the fracture surfaces of SiC sintered bodies. Scale bars, 50 µm.

We thank Mr T. Amano for scanning electron micrography work.

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Production of 'prompt' nitric oxide and decomposition of hydrocarbons in flames

The pollutant nitric oxide originates in combustion systems from nitrogen in the air or the fuel. Two processes have been identified whereby atmospheric N_2 produces NO. The Zel'dovich[1] mechanism has the rate-determining step $O+N_2 \rightarrow N+NO$ and is affected by oxygen atoms having concentrations[2,3] above those for equilibrium. Fenimore's 'prompt' scheme[4] is an attack on N_2 by some hydrocarbon fragment with a lifetime of several µs in the flame's reaction zone[5]. Probable[6] primary steps are

$$CH+N_2 \rightarrow HCN+N \quad (1)$$

$$C_2+N_2 \rightarrow 2CN \quad (2)$$

The oxidation of HCN and CN to NO has been discussed elsewhere[6,7]. We have burnt premixed H_2–O_2–N_2 flames and measured the 'prompt' NO resulting from trace additions of hydrocarbons to the burner supplies. We found that 'prompt' NO yield is proportional to the number of carbon atoms in the hydrocarbon molecule. We consider that the hydrocarbons fragment into species with one carbon atom, in, or close to, the preheating region. Thus formation of 'prompt' NO, soot, ions in flames, and CH radiation from hydrocarbons all show related behaviour.

We studied flames with temperatures of 1,900–2,350 K, burnt at atmospheric pressure on a Padley–Sugden[8] burner. They have a flat reaction zone and burned gases (diameter ~15 mm; length ~0.2 m), in which the velocity, temperature and composition profiles are those for plug flow. Without additive these flames are invisible, but with, say, 0.1% (by volume) of C_2H_2, a greenish-blue reaction zone is apparent 1 mm off the burner face. In certain conditions this reaction zone of the hydrocarbon burning in the H_2–O_2–N_2 flame lifts off the burner, and to avoid this the fastest burning hydrocarbons with up to three carbon atoms, that is, CH_4, C_2H_2 or CH_3CCH, were used. The addition of 1% of a hydrocarbon and removal of a little H_2 does not affect[9] the temperature or concentrations of H, OH or O. Flames were sampled through a water-cooled quartz probe into a chemiluminescent analyser to determine [NO] along the axis of the burned gases. These flames were too hot for NO_2 to exist in them.

Using C_2H_2 as additive (Fig. 1) the NO with no hydrocarbon can only originate from the Zel'dovich process, whereas the increase in [NO] with C_2H_2 added can be unambiguously ascribed to the 'prompt' mode of production[6]. In general, with hydrocarbon present, [NO] near the reaction zone is non-zero, and well downstream in the burnt gases all the curves of Fig. 1 are parallel, with the increments in [NO] being 'prompt' NO. The dependence of this 'prompt' NO yield on $[N_2]$ has been investigated by replacing some N_2 with argon, but maintaining the temperature constant. This has established that 'prompt' NO $\propto$ $[N_2]$ in the unburnt gases. A similar investigation demonstrated that the yield of 'prompt' NO is also proportional to the quantity of hydrocarbon present (Fig. 2). The linearity held irrespective of the hydrocarbon, as well as from 1,900 to 2,350 K and for fuel-rich and fuel-lean flames. The only exception appeared with cooler fuel-rich ($T \sim 1{,}900$ K, $[H_2]/[O_2]$ in unburnt gases > 5) flames, where plots as given in Fig. 2 did show initial linearity followed by a decrease in slope for hydrocarbon additions greater than 1%, giving a maximum and a subsequent drop in 'prompt' [NO]. This suggests that hydrocarbons can destroy NO, as well as produce it.

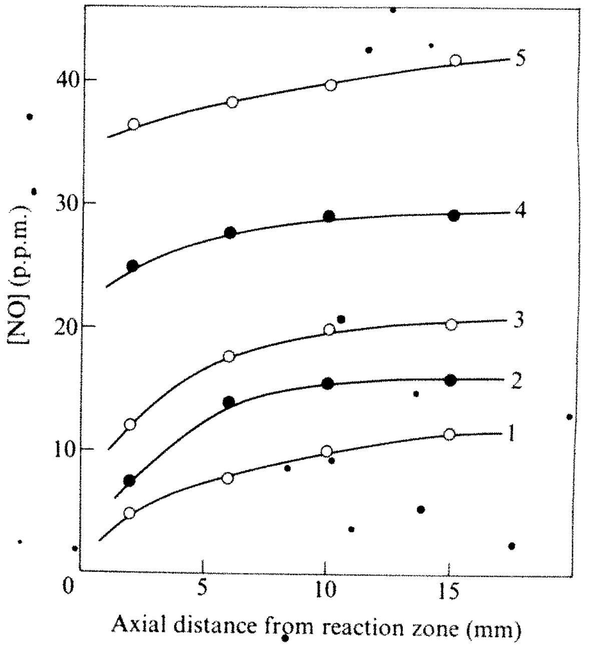

Fig. 1 Measured concentrations of NO along the axis of a flame ($[H_2]/[O_2]/[N_2] = 3.0:1.0:3.3$; temperature = 2,350 K) with various amounts of acetylene added. 1, 0%; 2, 0.98%; 3, 3.02%; 4, 4.43%; 5, 6.08%.

The efficiencies of CH_4, C_2H_2 and C_3H_4 at generating 'prompt' NO were compared (Fig. 3) for additions less than 1%. It is clear that the 'prompt' [NO] per N_2 and per hydro-

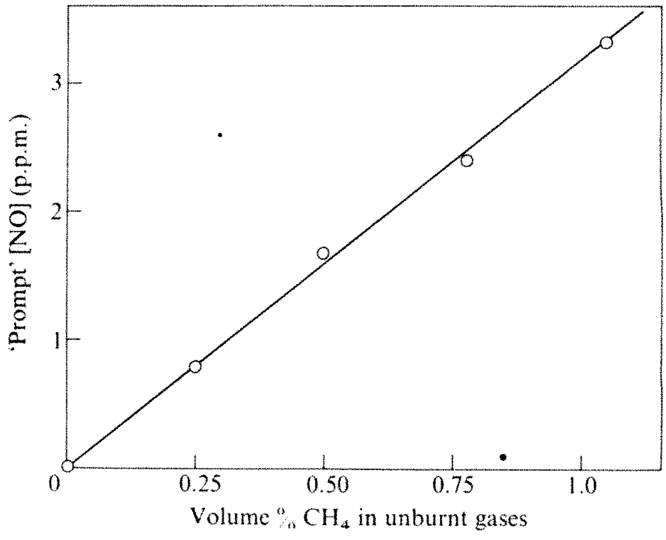

Fig. 2 Dependence of the yield of 'prompt' NO adjusted for any change in $[N_2]$ on the amount of methane added to a flame ($[H_2]/[O_2]/[N_2] = 3.0:1.0:5.7$; temperature = 1,900 K).

carbon molecule is proportional to the number of carbon atoms in a molecule of the additive. This was found to hold at different flame temperatures and compositions for fuel-rich systems. In fuel-lean flames the linearity was lost, with C_2H_2 more efficient per carbon atom than CH_4 or C_3H_4. A separate comparison between propyne and propane confirmed that both are equally effective promotors of 'prompt' NO in fuel-rich flames. Little variation of the 'prompt' NO (per carbon atom and per N_2 molecule) was detected with temperature or $[H_2]/[O_2]$ in the unburnt gases, provided the latter exceeded two. In contrast, the 'prompt' NO in O_2-rich flames decreased strongly for $[H_2]/[O_2]$ below two; when $[H_2]/[O_2] = 1$, there was always negligible 'prompt' NO. These observations establish that 'prompt' NO in fuel-rich systems is proportional to the number of carbon atoms present and independent of their origin. This rules out reaction (2), and others involving species containing two or more carbon atoms, as the primary step, because it is unlikely that say C_2 could come from CH_4 half as efficiently as from C_2H_2. Reaction (1) is apparently the most likely[6] primary step involving species with one carbon atom.

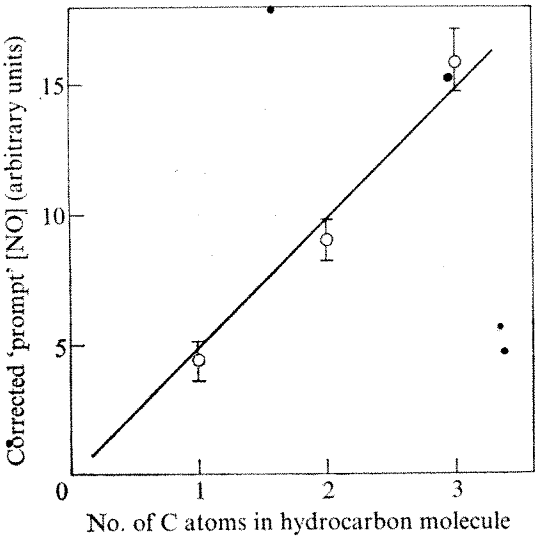

Fig. 3 The 'prompt' NO produced in a flame ($[H_2]/[O_2]/[N_2] = 3.0/1.0/3.3$; temperature = 2,350 K), per hydrocarbon molecule and per nitrogen molecule present in the burner supplies, plotted against the number of carbon atoms in the hydrocarbon.

The unburnt gases are known to pass through a pre-heating region before entering the reaction zone[5,10]. There is also an appreciable flux of hydrogen atoms into this region, because of their large concentration gradient in the reaction zone[2,9,10] and their immense diffusivity. One explanation then of the above is that CH arises from hydrocarbons undergoing a sequence of first-order decomposition reactions, with a common rate-determining step. If this holds for CH_4 and other hydrocarbons, the rate-determining step could be the conversion of CH_3 to CH_2 or CH_2 to CH. Also, since a large hydrocarbon gives as much CH per carbon atom as CH_4, the rupture of every carbon–carbon bond must occur before oxidation in the reaction zone interferes by producing stable products such as CO. A complete break-up of a hydrocarbon to CH_3 or CH_2 is possible given a significant inflow of hydrogen atoms and a pre-heating[5] of less than 1000 K. Now all the stripping reactions

$$\begin{aligned} CH_4+H &\rightarrow CH_3+H_2 \\ CH_3+H &\rightarrow CH_2+H_2 \\ CH_2+H &\rightarrow CH+H_2 \end{aligned}$$

are close to thermoneutral[11] and are exothermic if OH replaces H. Also

$$CH+H\rightarrow C+H_2$$

is exothermic by some 97 kJ mol^{-1}, so that a rate-determining step cannot be identified. In fact, the reverse of each of the above reactions is likely to be fast enough at say 800 K with the large $[H_2]$ for them to be partially equilibrated and a limited 'pooling' of CH_3, CH_2, CH and C to result. Of course, all these radicals are likely to react with O_2 rapidly enough to prevent a complete balancing of the above four reactions. As for the pyrolysis of a paraffin in the presence of H atoms, schemes are available[12] indicating that the likeliest products include CH_4, C_2H_4 and C_2H_2. Such schemes, with the sequence

$$\begin{aligned} H+RH &\rightarrow H_2+R \\ R &\rightarrow C_2H_4+R \\ H+C_2H_2 &\rightarrow C_2H_3 \\ H+C_2H_3 &\rightarrow C_2H_4 \\ H+C_2H_4 &\rightarrow C_2H_5 \\ H+C_2H_5 &\rightarrow 2CH_3 \end{aligned}$$

give the partial pool of species with one carbon atom, rapidly and first order in the hydrocarbon, before significant oxidation to CO occurs.

Since the rate constant of (1) is known[13], a crude check on the above conclusions is possible. Consider a flame with 1% of C_2H_2 and 45% of N_2 in its supplies. If 1 in 100 carbon atoms produce CH, which is taken to react in the preheating region every collision with O_2, then the 'prompt' NO is calculated to be 4.6 p.p.m. for the products of (1) going to NO. This compares with the observed 4.4 p.p.m. for 0.98% of C_2H_2 in Fig. 1. Such agreement indicates that the fragmentation of each fuel molecule into a pool of species with one carbon atom before significant CO production occurs is a real possibility.

This conclusion for fuel-rich flames is borne out by other work. Ferguson[14] concluded that soot formation is preceded by dissociation of every C–C bond. Bulewicz and Padley[15] confirmed that ions in flames originate[16] from $CH+O\rightarrow CHO^{+}+e^{-}$ with an efficiency proportional to the number of carbon atoms in a fuel molecule. Blades[17] reached the same conclusion and found the CH emission intensity to be equal on a per carbon atom basis for several hydrocarbons. Peeters *et al.*[18] also observed a first order dependence for the CH emission from various hydrocarbons including CH_4.

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Lower Cretaceous Lepidoptera

LEPIDOPTERA are rare in the fossil record and, until relatively recently, most fossil butterflies and moths had been found in Tertiary deposits. Records of Lepidoptera from earlier in the fossil record have been discounted[1]. The first evidence of a Cretaceous lepidopteran was Mackay's description[2] of the head of a caterpillar in amber (about 72 Myr BP). Kühne described micropterigid scales from Cretaceous resin (about 100 Myr BP) from West France[3], and several lepidopterous specimens have been found in Canadian and Siberian ambers of Cretaceous age (personal communication from A. Mutuura and A. Skalski). There is doubt about a much earlier record reported by Riek[4], who described two insects from Triassic beds in South Africa and placed them in the Paratrichoptera, which he considered a suborder of the Lepidoptera. (Evidence to suggest that this material is not lepidopterous will be published elsewhere.) Thus the four moths described here, which were found in Lebanese amber dating from at least 100 Myr BP, are the earliest indisputable lepidopterous specimens.

The amber was received from Professor Aftim Acra in Beirut. It was considered Aptian[5] or Neocomian[6] but must be at least 100 Myr BP, and Schlüter[7] has suggested an absolute age of 130 Myr BP. The moths (Fig. 1) have a wingspan of about 10 mm and have proved to be similar to extant species of Micropterigidae (Lepidoptera, Zeugloptera). They have been compared with recent species of Zeugloptera and with the primitive Dacnonypha, Agathiphagidae. The fossil moths have similarly shaped wings and wing scales, with abundant microtrichia on the wing membrane, comparable with recent micropterigids such as *Sabatinca* and *Micropterix*. The mouthparts have mandibles but no proboscis, and maxillary and labial palps similar to those of *Sabatinca* and *Micropterix*. Differences between the fossil and living species include the lack of ocelli and the apparent absence from the fossil forewing of a branch of the subcostal vein. The fossil moths have scales and an epiphysis on the foretibia, both of which are typical lepidopterous characters. Although the jugum cannot be seen in the fossils there is a small frenulum with four bristles similar to that of recent *Sabatinca*. A study of recent micropterigids shows that the fossil species is close to *Sabatinca* with which it has more characters in common than with *Micropterix*.

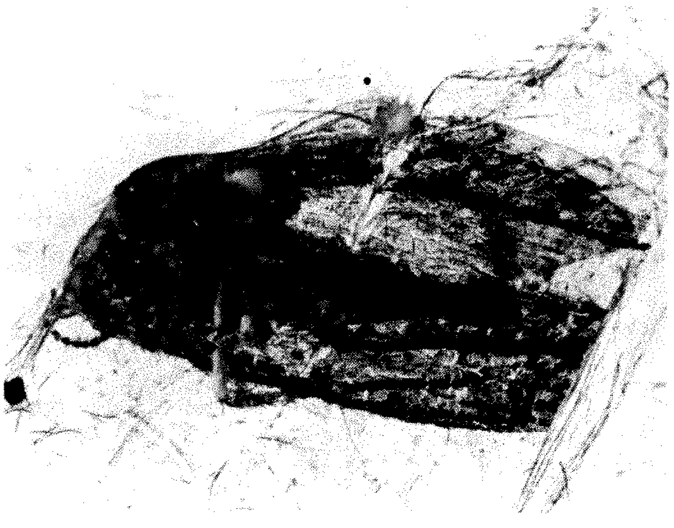

Fig. 1 Lower Cretaceous resin with lepidopterous inclusion. (Photo: British Museum Natural History.)

Re-examination of the holotype of *Micropterix pervetus* Cockerell[8] from Burmese amber of Miocene age (about 7 Myr BP) shows that this species should be transferred to *Sabatinca*. Similarly *Micropterix proavitella* Rebel[9], described from Baltic amber (Eocene/Oligocene, about 40 Myr BP) should also be transferred to *Sabatinca*. The genus *Sabatinca* has extant species in Australia, New Zealand and New Caledonia and its former distribution and origins can now be traced back through Burma in the Miocene, Northern Europe in the Eocene/Oligocene to probable origins in the Lower Cretaceous in the Lebanon. The Australasian species of *Sabatinca* are relicts of a former much wider distribution.

The newly discovered fossil provides evidence of Lepidoptera in the Lower Cretaceous but, because of its relatively specialised morphology, I propose an earlier date for the separation of the Lepidoptera from the Trichoptera–Lepidoptera stem-group than the Cretaceous origin suggested previously. We should now be looking for fossil evidence of Lepidoptera in the Jurassic. A fuller account of the morphology and relationship of these Lower Cretaceous fossil moths will be published elsewhere.

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Photoperiodic termination of diapause in spider mites

TERMINATION of diapause in the spider mite *Tetranychus urticae* is considered to depend solely on a period of chilling, the so-called cold rest[1-3]. The minimal period of chilling required to reactivate a certain percentage of the overwintering population may vary between populations of different geographic origin[4]. No unambiguous relationship, however, has been established between chilling period and latitude, for there may be appreciable differences in the minimal period of cold rest between strains from close localities[5]. The situation seemed more complex after Dubynina's[6] discovery that reactivation of a Russian strain of this species increased linearly with time when mites had been induced to diapause under the influence of long days, abrupt temperature changes and severe competition for food, while reactivation was cyclical when diapause had been induced by short days and relatively low temperatures. In the latter case maximal percentages of reactivating females were obtained after diapausing mites had been kept in cold storage for 40 and 75 d. Minimal percentages were obtained after 50 and 95 d. Reactivation was complete by about the 105th day of storage. Experiments in England, however, produced no evidence for cyclical reactivation in British strains of *T. urticae*[5]. Using a Dutch strain of *T. urticae*, which has a constant photoperiodic reaction and has been reared in the laboratory for more than 15 yr (ref. 7), we have now found that termination of diapause in this species depends not only on a period of chilling but on photoperiod as well.

Female mites were induced to diapause at 18 °C in a short-day photoperiod of 10L:14D and then stored at 4 °C, in either a long-day regime of 16L:8D or a short-day regime of 10L:14D. At regular intervals samples of about 50 diapausing mites were retrieved from this cold storage and their capacity to terminate diapause was tested by placing them on fresh bean leaves at three different temperatures (Fig. 1). Females were considered to have reactivated if, within 10 d, their colour had changed from the bright orange-red of the overwintering form to the yellowish-green of the 'summer' form, and feeding and oviposition had started. Photoperiod was constant during this test; mites which had been stored in the cold in the long-day regime were reactivated in the same long days, and mites which had spent their cold rest in short days were reactivated in short days of the same length.

The results (Fig. 1) show the influence of temperature on reactivation. With increasing temperatures during reactivation, the duration of the cold rest necessary to terminate diapause in a certain percentage of the test population becomes progressively shorter. Comparison of Fig. 1*a* with Fig. 1*b*, however, shows that at least during the first 2.5 months of diapause photoperiod had a far greater influence on reactivation than did temperature. For example, at 17 °C 50% of the mites terminated diapause in long-day conditions after a cold rest of only 8 d, but in short-day conditions termination of diapause in 50% of the test population was achieved only after a cold rest of 54 d. This difference in duration of cold rest, required to terminate diapause in 50% of the mites in the two photoperiodic regimes, is still 34 d at 20 °C and 28 d at 23 °C (Fig. 1). About 2.5 months after the beginning of diapause the photoperiodic sensitivity of diapausing females had apparently disappeared, for reactivation of the complete population succeeded from that moment onwards in short as well as long days. Thus termination of diapause in *T. urticae* is dependent not just on temperature, but, at least during the first few months, on photoperiod as well.

These experiments suggest that sensitivity to photoperiod remains in adult females of *T. urticae*, at least during the first few months of diapause, and that in long days and relatively high temperatures diapause can be terminated in part of the population without the intervention of a cold rest of even minimal duration.

Although even in the short-day regime, at all three temperatures tested, complete reactivation was achieved after a cold storage of about 70 d, experiments in both photoperiodic regimes were prolonged up to 120 d to look for any cyclicity in the pattern of reactivation. Reactivation proved to be linear, however, in accordance with the results obtained with British strains of *T. urticae*[5], and in contrast to Dubynina's[6] results.

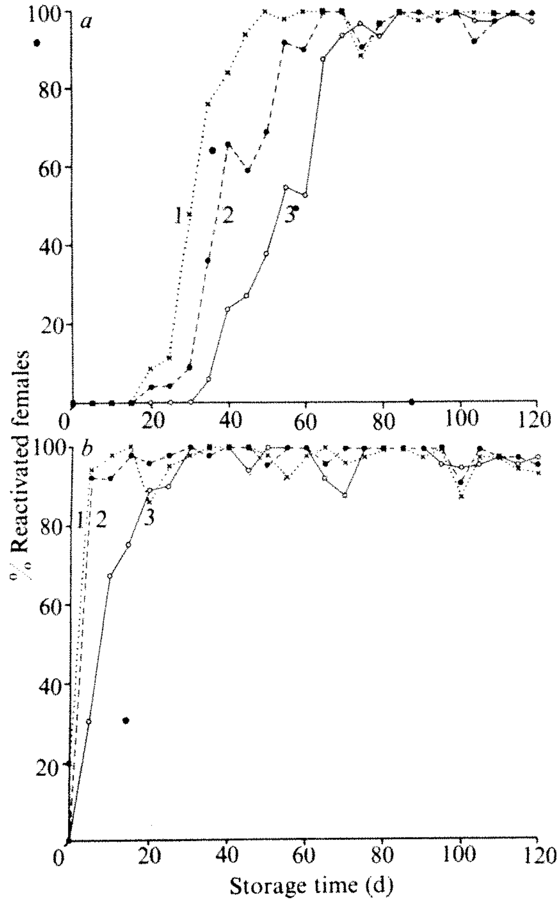

Fig. 1 Reactivation of diapause females of *T. urticae* at three different temperatures (1, 23 °C; 2, 20 °C; 3, 17 °C) in relation to the cold rest during which the mites were stored at 4 °C. *a*, Cold rest and reactivation in short days (10L:14D); *b*, cold rest and reactivation in long days (16L:8D).

Our results were obtained with a laboratory strain of *T. urticae*, and experiments with strains isolated from different localities in Europe are in progress to check the generality of the phenomenon of photoperiodic termination of diapause in this species. Some speculation, however, is possible about the ecological significance of the sensitivity of diapausing mites to photoperiod. It is conceivable that sensitivity during the early days of diapause tends to retain the diapause state during the autumn, when occasional warm days might break diapause in part of the population. According to our results the short days of autumn would prevent this breaking of diapause, while during winter diapause would be sustained and prolonged by prevailing low temperatures. In this sense, photoperiod would not function to terminate diapause when external conditions are favourable again, as in several insect species, but rather to protect the mites from becoming active again long before the season favourable for development and reproduction.

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Albinism in relation to competition in bamboo *Phyllostachys bambusoides*

THE ecological consequences of albinism in plants have not been investigated, although the phenomenon has been considered by physiologists, geneticists and cytologists[1–7]. Production of albino offspring generally reduces parental reproductive value, because albinos die after seed reserves are depleted, and thus do not reproduce. This negative effect can be moderated if a recessive allele for albinism confers increased fitness in the heterozygous state[8–13]. I present here a hypothesis that, in species producing dense stands of seedlings subject to interspecific competition, albino offspring can confer a competitive advantage to photosynthetic siblings; the reduction in parental fitness due to albinism may thus be less than predicted on the basis of reduced number of offspring. A test using bamboo (*Phyllostachys bambusoides*) supports this proposal.

Production of albino offspring by a given parent at a given time could increase growth and survivorship of non-albino members of the same cohort if the albinos shade interspecific competitors, but impose less intense intraspecific competition than would the same number of photosynthetic seedlings. As albinos deplete their seed reserves and die, adjacent photosynthetic seedlings should expand their crown and root systems to fill the space. Shading of competitors is thus maintained. Decomposition of albinos will release nutrients which can be absorbed by surrounding plants. The parental investment of resources in albino seeds may therefore be regained in part by photosynthetic offspring. The amount regained will depend on intensity of interspecific competition, as well as on rate of nutrient leaching from the soil.

This hypothesis was tested experimentally on Barro Colorado Island, Panama (9°10′N latitude, 79°51′W longitude, 50 m elevation). Green and albino seedlings of *P. bambusoides* Sieb. and Zucc. (Graminae), a monocarpic bamboo introduced from Asia, were grown alone and in competition with seedlings of *Sida acuta* Burm. f. (Malvaceae), a very widely distributed pantropical weed. The bamboo occurred as a dense clone approximately 5 m wide by 10 m long, with stalks of approximately 15 m. The bamboo was located on the edge of a large

Table 1 Mean dry weight per individual and percentage mortality for experimental plants

	Treatment no.									
	1	2	3	4	5	6	7	8	9	10
Number of plants per pot*										
Albino bamboo	0	0	0	0	0	24	48	48	0	0
Green bamboo	24	48	96	24	48	24	0	48	0	0
Sida	0	0	0	48	48	48	48	0	48	96
Green bamboo†										
Mean dry weight (g × 10^{-4})	212	206	142	164	146	203	—	196	—	—
95% confidence limit	14.7	13.4	9.0	20.9	12.8	21.5	—	21.3	—	—
% mortality	6	8	10	8	13	0	—	11	—	—
Sida										
Mean dry weight (g × 10^{-4})	—	—	—	70	71	67	75	—	78	64
95% confidence limit	—	—	—	6.6	5.4	5.0	6.6	—	6.8	6.0
% mortality	—	—	—	0	2	45‡	3	—	3	19

* 24 plants per pot = 1,323 plants per m^2; 48 plants per pot = 2,645 plants per m^2; 96 plants per pot = 5,290 plants per m^2.

† Median tests for green bamboo dry weights (d.f. = 1 in all cases): treatment 1 vs 4, $\chi^2 = 7.66$, $P < 0.01$; 1 vs 6, $\chi^2 = 0.01$, not significant; 4 vs 6, $\chi^2 = 9.46$, $P < 0.01$; 1 vs 3, $\chi^2 = 11.06$, $P < 0.001$; 2 vs 8, $\chi^2 = 0.46$, not significant; 3 vs 8, $\chi^2 = 14.70$, $P < 0.001$; 3 vs 6, $\chi^2 = 11.44$, $P < 0.001$.

‡ 65% in one replicate, 25% in the other; median test for green bamboo dry weights within treatment 6, replicate 1 vs replicate 2, $\chi^2 = 0.33$, d.f. = 1, not significant.

clearing. It produced abundant seed between January 1975 and July 1976, and then died. Dense stands of seedlings appeared around the clone during the wet seasons of these years (May–December). Average seedling density on 19 May 1976 was 1,549±1,089 seedlings per m^2; 25.4±3.21% of all seedlings were albino. This ratio was also demonstrated during the wet season of 1975 (Carol Augspurger, personal communication). A one-to-three ratio of albino to green seedlings suggests that albinism is determined by a recessive gene. Average leaf area per seedling on 19 May was 1.4±0.32 cm^3 for green bamboo, and 1.4±0.40 cm^2 for albinos. *Sida acuta* was the only species producing abundant seedlings of uniform size in the same habitat as *Phyllostachys* at the time of the study. It is an herbaceous perennial growing to a maximum height of approximately 2 m in clearings and on forest edges.

Seedlings of both species were excavated on 6 May 1976. Mean height of excavated seedlings was 7.2±1.15 cm for green bamboo, 6.3±1.16 cm for albinos and 1.3±0.19 cm for *Sida*. Bamboo seedlings were approximately 2 weeks old at the time of collection, and all retained connections to seeds which seemed to contain endosperm. *Sida* seedlings were in the cotyledon stage and had not yet produced mature leaves. Seedlings were transplanted with bare roots to soil-filled clay pots 15.2 cm in diameter and 12.3 cm deep. Soil was a Frijoles clay[14], collected from a site near the bamboo clone, and thoroughly mixed. Seedling densities in each treatment are given in Table 1. There were no significant between-treatment differences in height for a given type of seedling at the beginning of the experiment. Height was linearly correlated with dry weight in a series of non-experimental seedlings: for green bamboo $r = 0.81$, $P < 0.01$, $n = 44$; for albino bamboo $r = 0.88$, $P < 0.01$, $n = 44$; for *Sida* $r = 0.84$, $P < 0.01$, $n = 45$. Each experimental treatment was replicated twice. The two replicates were initiated at different times during the planting period, to avoid between-treatment differences due to time of planting. Lack of sufficient *Sida* seedlings of uniform size prevented greater replication. A carefully measured grid system was used to ensure uniform spacing of seedlings. In treatments containing more than one seedling type, seedlings were arranged in regular alternating sequences (ABAB, AABAAB, ABACABAC, and so on) determined by relative proportions of each type.

Pots were placed in a growing house with walls and roof of insect screening. A clear plastic sheet was used to eliminate rain. The growing house was in a clearing and could receive direct insulation from mid-morning until mid-afternoon. The experiment took place during the wet season, when cloudy weather was predominant. Each pot received 60 ml of untreated water from Lake Gatun every other day. Location of each pot relative to others was randomised and changed every other day to avoid position effects. All seedlings which died during the first 2 d after planting were replaced. Replacement of albino seedlings was continued for 2 weeks after planting. Initial mortality of albinos seemed to result primarily from damage to seedling-seed connections during planting and was evenly distributed among treatments. Marked albino plants in the field did not die during this period. The experiment was terminated after 37 d, when all albinos had died. Marked albinos in the field had also died by this time. Dead seedlings decayed very rapidly due to fungal attack. Seedlings were cut at ground level, dried to constant weight at 70 °C, and weighed.

Results (Table 1) are consistent with the hypothesis. Green bamboo seedlings grown in pure culture at three densities showed lowest mean dry weight per plant at highest density. This demonstrates intraspecific competition, a necessary condition for the hypothesis. Green bamboo grown at intermediate density with an equal number of albinos, however, did not differ significantly in dry weight from green bamboo in pure culture. Thus, albino and green bamboo do not seem to compete. Green bamboo grown at low and intermediate densities in the presence of *Sida* had significantly lower mean dry weight per plant than did plants grown in pure culture at the same densities, demonstrating interspecific competition. When green and albino bamboo were grown together in the presence of *Sida*, however, mean dry weight per green bamboo plant was not significantly different from that for controls. Mortality of *Sida* seedlings was much greater in the presence of a mixed green–albino population than when grown only with green bamboo or only with albino bamboo. Differences among means for *Sida* dry weight were not statistically significant. Mortality of green bamboo was too low to permit meaningful comparisons among treatments.

This experiment provides the first evidence that plants with a lethal genotype can confer a competitive advantage to siblings with non-lethal genotypes. The primary result of this competitive effect in natural populations might be a reduction in the intensity of selection against albinism; the fitnesses of parents producing partially albino cohorts could remain lower than fitnesses of parents producing entirely photosynthetic cohorts despite the competitive effect. To be of adaptive value, the benefits of improved competitive ability would have to outweigh the energetic cost of producing seeds with albino genotypes, the cost of reduced number of viable offspring, and potentially, the cost of reduced genetic variability among viable offspring due to reduced numbers. Surveys are planned to determine if the phenomenon is likely to occur in natural populations. Regardless of its importance in natural conditions, agricultural applications of this effect should be considered.

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Increased homoeologeous chromosome pairing in *Hordeum vulgare* x *Triticum aestivum* hybrids

WHEAT and barley are the most important cereals in the agriculture of temperate zones. Although both genera belong to the same tribe, Triticeae, apparently successful crosses between wheat and barley have been reported recently[1,2]. I report here the production, morphology and cytology of F_1 hybrids of *Hordeum vulgare* L. cv Betzes × *Triticum aestivum* L. cv Chinese Spring.

The emasculation of barley, the maternal parent, pollination by wheat pollen, application of the gibberellin GA_3 to pollinated florets and subsequent excision and culture of hybrid embryos on a nutrient medium were performed as described before[3]. From the 372 florets pollinated, 44 embryos were obtained of which four seedlings (9.0%) survived. Examination of root-tip squashes revealed $2n=28$ chromosomes, the expected number in such hybrids. Further confirming evidence for hybridity was obtained from studies of esterase patterns of leaf tissue in the hybrids. The hybrid pattern was a combination of the differential parental bands. No hybrid bands were detected (Fig. 1*b*).

The hybrid plants were as vigorous as the parents and had foliage of characteristic light green colour. Unlike either parent with the exception of colour and plant height, which was intermediate to the parents, the phenotype was typically paternal. The wheat-like pubescent auricles were evident from the early seedling stage to maturity. The spikes differed slightly from those of wheat by being somewhat longer and more lax with awnletted lemmas (Fig. 1*a*). Pollen grains of the hybrid were empty and shrivelled, the anthers were minute and indehiscent, and the plants were completely male sterile. A small number of seeds was obtained by pollinating the hybrid with wheat pollen, indicating some degree of female fertility.

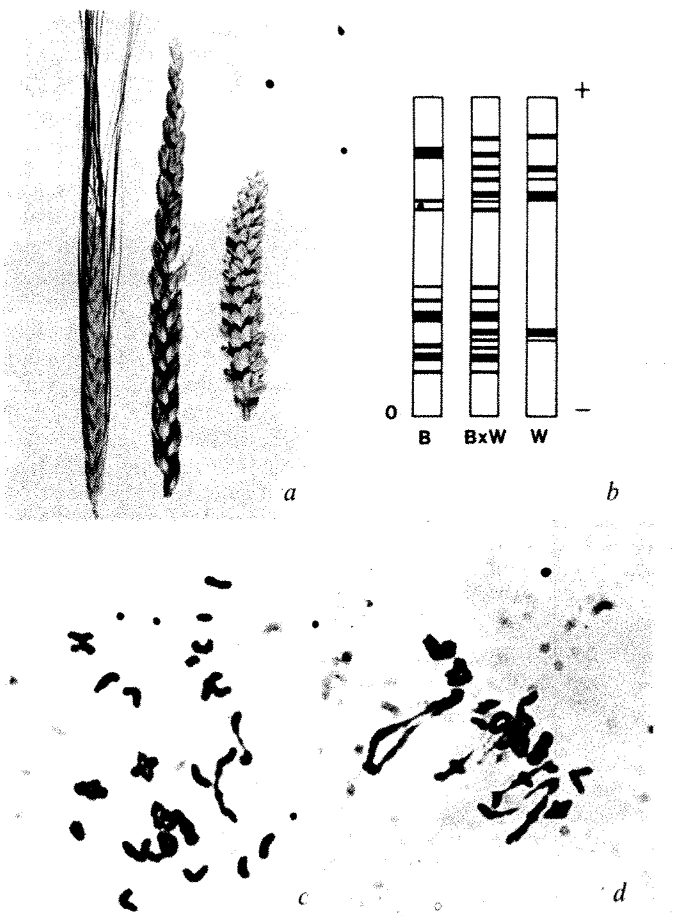

Fig. 1 *a*, Spikes of (left to right) barley; barley–wheat hybrid; wheat. *b*, Diagram of acrylamide gel electrophoresis of esteresa isozymes from leaf tissue of: barley (B); barley–wheat hybrid (B+W); wheat (W); *c*, metaphase I in barley–wheat hybrid showing one trivalent, 6 bivalents, 18 univalents (6 dyads and 12 monads) the latter indicative of equational division (×428); *d*, Metaphase I in barley-wheat hybrids showing one hexavalent, one quadrivalent, eight bivalents and two univalents (×428).

Meiotic behaviour in the four barley–wheat hybrids was similar; therefore the data were combined (Table 1). Meiotic configurations at metaphase I in individual cells ranged from 21 univalents with a considerable amount of equational division at anaphase I; to cells with five or more ring bivalents (Fig. 1*c*) or a combination of complex configurations such as quadrivalents and hexavalents (Fig. 1*d*) to give mean frequencies per cell of 19.88 univalents, 1.72 bivalents, 0.40 trivalents, 0.12 quadrivalents and 0.03 hexavalents.

A portion of the chromosome pairing in the barley–wheat hybrids would be expected to be of the autosyndetic type not unlike that observed in the haploids of both

Table 1 Mean chromosome pairing at first metaphase of meiosis in F_1 hybrids from crosses between *H. vulgare* and *T. aestivum* compared to haploids of the two species and other intergeneric hybrids

	Configurations							
Genotype	I	II	III	IV	V	VI	Xta.per cell	No. of cells
H. vulgare × *T. aestivum*	19.88	1.72	0.40	0.12	—	0.03	1.82	422
T. aestivum—haploid[4]	18.84	1.05	0.02	—	—	—	—	750
H. vulgare—haploid[5]	6.90	0.04	—	—	—	—	—	589
Sum of barley and wheat haploids	25.74	1.09	0.02	—	—	—	—	
T. aestivum × *T. longissimum*[6]	24.56	1.69	0.02	—	—	—	1.75	—
T. aestivum × *T. tripsacoides*[7]	—	—	—	—	—	—	1.51	50

species. The number of paired chromosomes in the barley–wheat hybrids, however, exceeded the sum total of paired chromosomes in haploid barley and haploid wheat (Table 1). Genotypes in diploid wheat species such as *T. tripsacoides*[7] (*Ae. mutica*) and *T. longissimum*[6] suppress the effect of diploidising genes in wheat to variable degrees. A mean chiasmata per cell of 1.82 in the barley × wheat hybrids indicates that the barley genotype may also suppress the diploidising system in Chinese Spring similar to that of the weak suppressors reported in *T. tripsacoides*[7] and *T. longissimum*[6] which showed 1.51 and 1.75 chiasmata per cell, respectively, in hybrids with Chinese Spring. The data suggest that in barley as in some diploid *Triticum* species a locus (or loci) has evolved that permits some homoeologous pairing in hybrids with unrelated species. It remains to be determined whether there are extensive intraspecific differences in the genus *Hordeum*, as there are in certain species of the genus *Triticum*, for genes that relax the suppression of homoeologous pairing of wheat chromosomes. The infrequent occurrence of a heteromorphic bivalent at metaphase I suggested that there may be some homology between chromosomes of barley and wheat, providing an alternative explanation for the increased pairing in the hybrid above the sum of the two haploids. The application of Giemsa staining techniques for meiotic chromosomes in barley–wheat hybrids could help to solve this problem.

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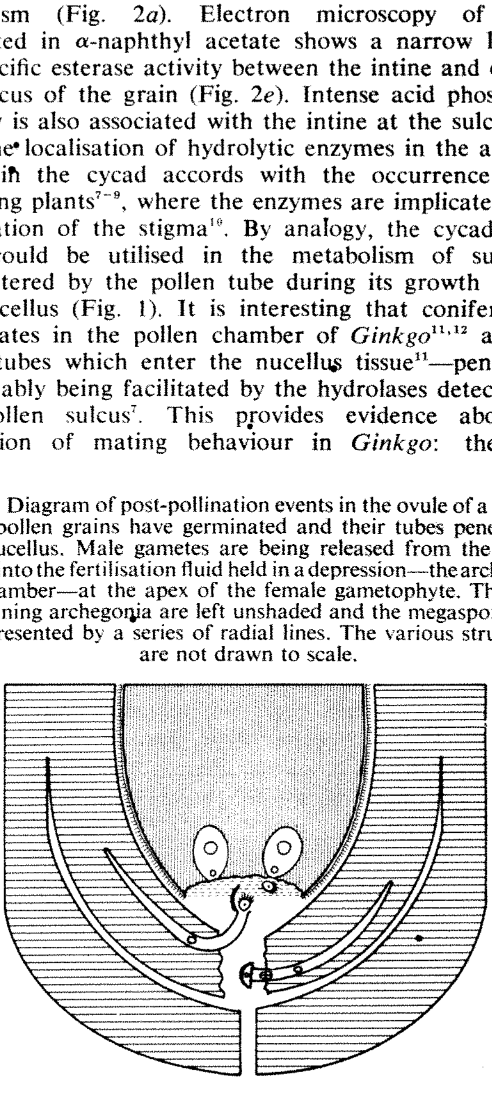
Detection in primitive gymnosperms of proteins and glycoproteins of possible significance in reproduction

THE modern cycads are the remnant of a gymnosperm group that has been in existence for at least 200 Myr[1]. Cycads are dioecious, with separate male and female plants, and fertilisation is effected by motile male gametes—so-called zooidogamous reproduction. Zooidogamy is found also in *Ginkgo*, another dioecious gymnosperm of considerable antiquity[1]. Reproduction in all other gymnosperms and in flowering plants is siphonogamous. Here fertilisation occurs through a pollen tube which conveys the non-motile male gamete to the egg. A pollen tube is produced in the zooidogamous gymnosperms, but it is not involved in gamete transfer; it simply anchors the male gametophyte to the ovule during gametogenesis (Fig. 1). The ripe male gametes are released to swim freely in a pool of fluid bathing the openings of the egg-containing archegonia (Fig. 1). It is not known how the gametes that carry out fertilisation are selected in these plants. Dioecism ensures outcrossing, but there is presumably some form of intraspecific screening. The male gametophyte or male gamete could, for example, identify itself to the parent of its prospective mate, or the male gamete to the egg cell at the threshold of syngamy. I report here the occurrence in cycads of proteins and glycoproteins at sites in the pollen grain and ovule where they might be involved in reproduction.

Nonspecific esterase activity was detected in cryostat sections of *Macrozamia reidlei* pollen slurries using 5-bromoindoxyl acetate and α-naphthyl acetate as substrates, the latter in a coupling reaction with fast blue B salt[2]. For electron microscopy, the pollen was incubated in the reaction mixture before glutaraldehyde fixation and osmium post-fixation. Controls were run without substrate in all tests. Acid phosphatase activity was localised in freeze-sectioned pollen by Gomori's lead salt method[3] and the controls were incubated in the reaction mixture containing 0.01 M sodium fluoride. Nonspecific esterase activity was localised in cryostat sections of fresh *Cycas thouarsii* ovules by the indigogenic method. Acid phosphatase was investigated in ovules in the same way as for the pollen. Concanavalin A (con A)-binding sites in the megaspore wall of *C. thouarsii* were revealed ultrastructurally in glutaraldehyde-fixed ovules by coupling with horseradish peroxidase (HRP) and diaminobenzidine (DAB)[4], and by fluorescence microscopy in fresh, frozen sections with con A labelled with fluorescein isothiocyanate (con A-FITC, L'Industrie Biologique Française). The con A in the controls for both methods contained 0.2 M α-D-methyl mannoside[5]. Homogenates of *C. siamensis* megagametophytes were prepared in buffered saline, filtered and dialysed against water. The dialysate was concentrated and the concentrate was tested for ability to agglutinate trypsinised, human O+ erythrocytes and analysed by sodium dodecyl sulphate (SDS)–polyacrylamide electrophoresis[6] after dissociation of the proteins in mercaptoethanol and SDS. The gels were stained with Coomassie blue for proteins and the periodic-acid-Schiff technique for carbohydrates.

Proteins with enzymatic properties are contained in the pollen grain wall and the cytoplasm of the male gametophyte in *M. reidlei*. The indigogenic method reveals discrete granules of nonspecific esterase activity in the tube cell cytoplasm (Fig. 2*a*). Electron microscopy of pollen incubated in α-naphthyl acetate shows a narrow layer of nonspecific esterase activity between the intine and exine at the sulcus of the grain (Fig. 2*e*). Intense acid phosphatase activity is also associated with the intine at the sulcus (Fig. 2*c*). The localisation of hydrolytic enzymes in the apertural intine in the cycad accords with the occurrence in the flowering plants[7–9], where the enzymes are implicated in the penetration of the stigma[10]. By analogy, the cycad hydrolases could be utilised in the metabolism of substrates encountered by the pollen tube during its growth through the nucellus (Fig. 1). It is interesting that conifer pollen germinates in the pollen chamber of *Ginkgo*[11,12] and produces tubes which enter the nucellus tissue[11]—penetration presumably being facilitated by the hydrolases detectable at the pollen sulcus[7]. This provides evidence about the regulation of mating behaviour in *Ginkgo*: the ovule

Fig. 1 Diagram of post-pollination events in the ovule of a cycad. Two pollen grains have germinated and their tubes penetrated the nucellus. Male gametes are being released from the lower grain into the fertilisation fluid held in a depression—the archegonial chamber—at the apex of the female gametophyte. The egg-containing archegonia are left unshaded and the megaspore wall is represented by a series of radial lines. The various structures are not drawn to scale.



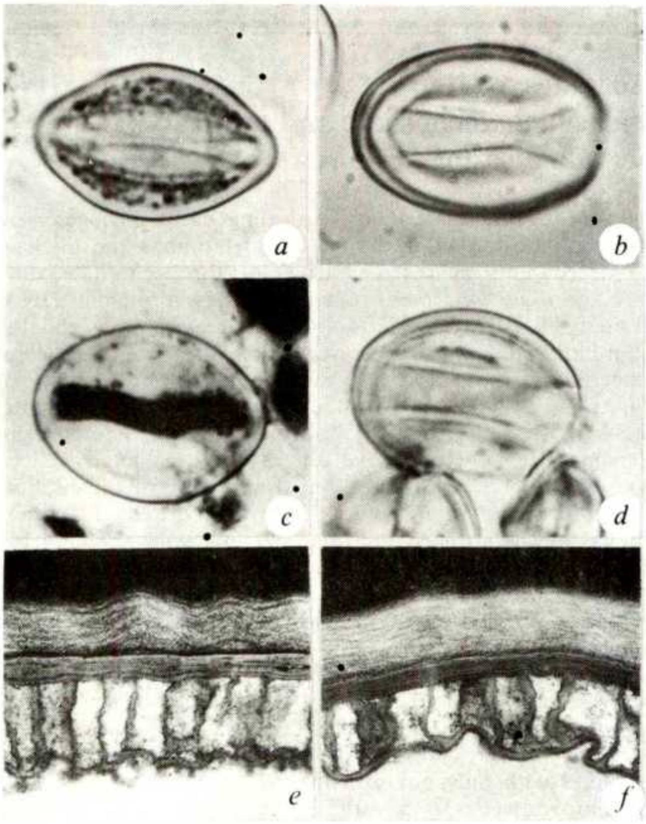


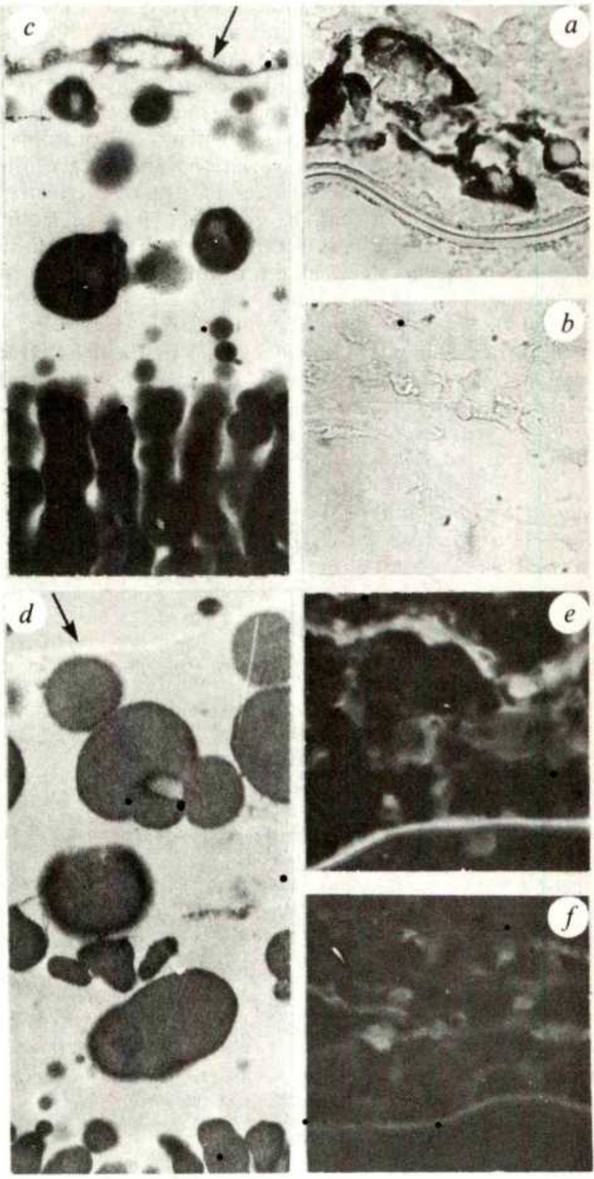
Fig. 2 Pollen of the cycad *M. reidlei*. *a*, Nonspecific esterase activity (× 1,000). *b*, Esterase control (× 1,000). *c*, Acid phosphatase activity (× 1,000). *d*, Acid phosphatase control (× 1,000); *e*, Electron microscope localisation of nonspecific esterase activity between the exine and intine at the sulcus (× 20,000). *f*, Control for (*e*) (× 20,000).

possesses no capacity for suppressing germination or frustrating the growth of the pollen tube in wide pollinations.

The female gametophyte in the zooidogamous gymnosperms is contained by a multilayered megaspore wall, comparable with the flowering plant pollen wall[13]. During development material derived from autolysis of the sporophyte cells immediately external to the gametophyte accumulates in the chambered outer layer (exine) of both walls. In the flowering plants the source cells comprise the tapetum[10,14,15] and in the gymnosperm ovule they are the inner layers of the nucellus[13]. There is a degree of chemical conformity between the walls and sporophytic loads in the two groups. Strong nonspecific esterase activity, indubitably of sporophytic origin, is found in the residue resulting from the breakdown of the nucellus cells in *C. thouarsii* ovules (Fig. 3*a*). There is no detectable acid phosphatase activity. Esterases, but not acid phosphatase, occur in the pollen exines of many flowering plants and their activity is judged a characteristic marker for the transfer of proteins from the tapetum to the pollen grain surface[16].

My experiments have demonstrated a carbohydrate moiety in the cycad megaspore wall able to bind con A. Electron microscopy shows that in *C. thouarsii* con A is bound specifically to the surface of an electron-lucent wall component incorporating sporopollenin droplets derived from the nucellus cells (Fig. 3*c*). Moderate to strong FITC fluorescence is emitted by the degenerating nucellus cells and by the megaspore exine in freeze-sectioned ovules treated with con A–FITC (Fig. 3*e*), the fluorescent material on the wall corresponding to the con A-binding layer observed in con A–HRP coupling. The specificity of con A towards oligosaccharides with terminal glucose and mannose residues is well authenticated[17], and acceptor sites for it are present in the pollen wall[18] and stigma surface pellicle—the receptor site for pollen-borne recognition molecules[19–21]—in flowering plants[22,23]. If the pellicle is coated with con A, penetration of the stigma is prevented in compatible pollinations[23], suggesting that the con A sites are necessary to one level of acceptance response in the reproduction of flowering plants. This cannot apply in the cycad megaspore, because above the archegonia the wall is dispersed before the male gametes are released (Fig. 1). But if this process is contingent on necrosis of the female gametophyte tissue to form the archegonial chamber (Fig. 1), the fertilisation pool could hold in solution molecules from both components. A concentrated extract of megagametophyte plus wall of *C. siamensis* haemagglutinates trypsinised human erythrocytes (Fig. 4*a*), signifying the presence of a phytohaemagglutinin or lectin[24]. Electrophoretic analysis of the extract reveals nine protein bands (Fig. 4*c*) three of which give a reaction for carbohydrates (Fig. 4*d*). In the conditions

Fig. 3 Ovule of cycad *C. thouarsii*. *a*, Nonspecific esterase activity in the degenerating nucellus cells. The megaspore wall is the refractive structure towards the bottom of the picture (× 500). *b*, Control for (*a*) (× 500). *c*, Megaspore wall after incubation in con A and coupling with HRP. Con A is bound to the surface of the electron-lucent wall component; the resultant DAB reaction product is indicated by the arrow (× 20,000). *d*, Con A control. The white line indicated by the arrow is the surface of the electron-lucent wall layer (× 20,000). *e*, Con A binding sites revealed with con A–FITC. Strong fluorescence is emitted by the degenerating nucellus cells and megaspore exine (× 300). *f*, Control for con A–FITC test. The section shows only autofluorescence (× 300).



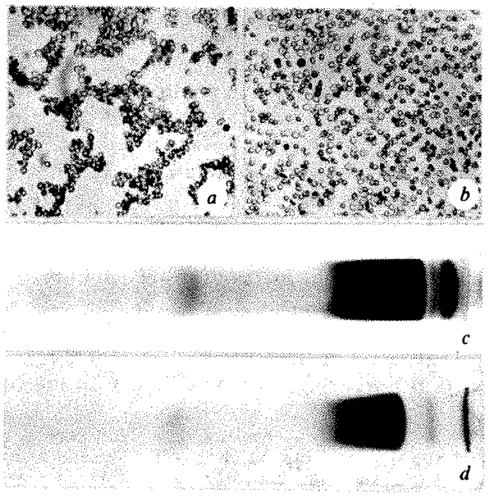


Fig. 4 Agglutination of trypsinised human erythrocytes produced by a saline-held extract of the megagametophyte and megaspore wall of the cycad *C. siamensis*. *b*, Control preparation for the agglutination test. Cells were treated with 0.15 M saline instead of extract. *c*, Protein bands in an SDS–polyacrylamide gel of the extract. *d*, Complementary gel stained for carbohydrates.

of the run these are likely to mark carbohydrates of glycoproteins.

This finding offers an intriguing possibility for the control of intraspecific mating in the primitive, zooidogamous gymnosperms. It has been suggested that lectins in plants have a defence function and may also be involved in the determination of self-recognition between cells[25]. Lectins interact with animal cells[24] and with plant cell protoplasts[26] and can detect intraspecific variations in surface oligosaccharides[24]. As a consequence of these properties a function in recognition can be envisaged for the lectin in the gymnosperm ovule: as a constituent of the fertilisation pool it could be involved in discrimination between male gametes.

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Isozymes of a tunicate and a cephalochordate as a test of polyploidisation in chordate evolution

IN view of the uncertainty regarding early phylogeny of the chordates[1,2], Atkin and Ohno[3] determined the nuclear DNA contents of representatives of three chordate subphyla. *Ciona intestinalis* (Tunicata) has a diploid DNA content of 0.42 pg, while *Amphioxus lanceolatus* (Cephalochordata) contains 1.2 pg—very close to the 1.4 pg considered as the basic vertebrate value[4,5]. Assuming a tunicate-like ancestry of the cephalochordate-vertebrate stock[6–9], Ohno[10] proposed that the threefold difference in genome size between *Ciona* and *Amphioxus* might reflect the occurrence of at least one round of polyploidisation preceding vertebrate evolution. To test further this hypothesis we determined the number of genes coding for various enzymes in *C. intestinalis* and *Branchiostoma* (*Amphioxus*) *lanceolatum*. Only few genetically interpretable isozyme data from *B. lanceolatum* have been reported[11,12]. An isozyme study on a related species, *B. moretonensis* has been published recently[13]. Systematic isozyme investigations in tunicates have not been reported before. We report here that *C. intestinalis* and *B. lanceolatum* have identical gene numbers for homologous enzymes with only one exception.

Comparative isozyme studies have proved a powerful tool in substantiating ancestral diploid–tetraploid relationships, for example in teleostean fish[14] and various examples of gene duplication in mammals including man have been taken as support of the idea of ancestral polyploidy in this taxonomic category[15,16]. Thus, if relative to *Ciona*, *Branchiostoma* represents an ancestrally polyploid organism, it is conceivable that it still possesses an increased number of functional gene loci. Table 1 summarises the results of our electrophoretic investigations. Data from 10 of the 12 isozyme systems analysed were interpretable in each, *Ciona* and *Branchiostoma*. Of these, nine lend themselves to a direct comparison: five enzymes seem to be encoded for by a single gene locus in *Ciona* as well as *Branchiostoma*; one system (AAT) is clearly encoded for by two gene loci in both organisms, whereas the number of loci underlying the multiple zones of superoxide dismutase activity remains unclear, since only the slowly migrating zone resolved into sharp bands. The NAD-MDH pattern observed in *Ciona* is best interpreted by the assumption of two loci while in *Branchiostoma* a third, additional NAD-MDH locus is present. Multiple zones of esterase activity were seen when 4-methylumbelliferyl-acetate was substrate. The dimeric enzyme seen in human tissue in these conditions[17] appeared in *Branchiostoma* only.

Our data do not support the polyploidisation hypothesis of Ohno, since in one isozyme system only (NAD-MDH) *Branchiostoma* is endowed with a higher number of genes than *Ciona*. It should be emphasised, however, that our data do not contradict a polyploidisation hypothesis. In the course of regaining diploidy, a tetraploidised organism tends to functionally eliminate the duplicate genes by accumulation of 'forbidden' mutations, unless some special selection pressure favours their maintenance[5,14,18,19]. *Branchiostoma*, if anciently polyploid, may already have silenced the majority of duplicated genes. It should be mentioned that with respect to the ribosomal DNA content, apparently subject to especially drastic change during evolution[20], there is a 1 : 6 ratio between *Branchiostoma* and *Ciona*[21]. The failure to detect more duplicated genes in *Branchiostoma* using the whole body extracts from adult animals may also be due to a possible differential activity of gene duplicates during development and/or in different

Table 1 Analysis of electrophoretic data of isozyme studies in *C. intestinalis* and *B. lanceolatum*

		C. intestinalis					*B. lanceolatum*				
Enzyme		No. of animals investigated	No. of alleles found	Allele frequencies*			No. of animals investigated	No. of alleles found	Allele frequencies*		
NAD-MDH	fast	21	2	0.33	0.66		100	2	0.01	0.99	
1.1.1.37	slow	21	3	0.03	0.94	0.03	100	3	0.03	0.96	0.01
	very slow						100	2	0.01	0.99	
NADP-IDH		13	3	0.07	0.73	0.20	very faint staining				
1.1.1.42											
6–PGDH		9	5	0.33	0.28	0.17	very faint staining				
1.1.1.44				0.17	0.06						
Catalase		20	2?				13	3?			
1.11.1.6											
Peroxidase		no staining					13	3	0.04	0.92	0.04
1.11.1.7											
Superoxide dismutase	fast	23	variable				very faint staining				
1.15.1.1	slow	23	2	0.20	0.80		13	2	0.73	0.27	
AAT	fast	14	3	0.20	0.55	0.25	14	3	0.04	0.32	0.64
2.6.1.1	slow	14	2	0.10	0.90		14	2	0.04	0.96	
PGM		27	2 ?				14	5	0.11	0.32	0.11
2.7.5.1									0.29	0.18	
Methylumbelliferylesterase	fast	12	3	0.17	0.67	0.17	17	variable			
	slow	12	variable				17	variable			
3.1.1.1	very slow (dimeric)						17	3	0.09	0.88	0.03
Adenosinedeaminase		14		unvarying			14	5	0.25	0.07	0.54
3.5.4.4									0.07	0.07	
Glyoxalase I		8	3	0.06	0.81	0.13	14	2	0.18	0.82	
4.4.1.5											
PGI		11	6	0.05	0.23	0.05	95	2	0.98	0.02	
5.3.1.9				0.36	0.14	0.18					

All animals were caught in the North Sea at Helgoland. *C. intestinalis* individuals were freed from their tunics before further preparation. All animals were homogenised in 0.01 M phosphate buffer, *p*H 7.4, containing 10^{-4} M $MgCl_2$, in a glass–glass homogeniser, and the 20,000*g* supernatants were subjected to horizontal starch gel electrophoresis. All enzymes were analysed in gels consisting of 14% starch in 0.08 M Tris/0.03 M citrate buffer, *p*H 8.0 (bridge: 0.223 M Tris/0.068 M citrate buffer, *p*H 8.0) except esterase, adenosinedeaminase glyoxalase and superoxide dismutase (the latter in the case of *Branchiostoma* only); these enzymes were investigated in 13% starch in 0.006 M potassium phosphate buffer, *p*H 7.4 (bridge: 0.375 M. potassium phosphate buffer, *p*H 7.4). Staining was performed by conventional methods[17, 24–26]. Attempts to stain lactate dehydrogenase, glucose-6-phosphate dehydrogenase, sorbitol dehydrogenase and alcohol dehydrogenase were either unsuccessful or yielded results too poor for genetic interpretation. MDH, maleate dehydrogenase; IDH, isocitrate dehydrogenase; 6–PGDH, 6–phosphogluconate dehydrogenase; AAT, aspartate amino transferase (GOT); PGM, phosphoglucomutase; PGI, phosphoglucose isomerase.

*Most cathodal variant first.

organs[5,12]. Furthermore, as Ohno[5] has pointed out, duplicated genes may have been altered with respect to kinetic properties, *p*H and temperature optima, and substrate specificity. Thus their detection by standard electrophoretic and staining procedures may be prevented.

An alternative to the polyploidisation hypothesis is that the common ancestor of tunicates—cephalochordates and vertebrates already possessed a DNA content similar to the basic present-day vertebrate value, and that during the evolution and specialisation of the tunicates an appreciable part of the nuclear content was lost. Loss of nuclear DNA content concomitant with specialisation has been noted by Hinegardner[22].

As pointed out by Ohno[10] a further alternative to the polyploidisation hypothesis relies on the assumption that the genome size increase in *Branchiostoma* relative to *Ciona* (or its ancestor) was due to accumulation of repetitious DNA. It has been shown[23] that the *Ciona* genome contains approximately 30% repetitive DNA, but the genome composition of *Branchiostoma* is not known. Its analysis might clarify chordate interrelationships.

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Similarity of the general designs of protochordates and invertebrates

THE protochordate amphioxus is clearly differentiated from the invertebrates by most taxonomic criteria[1,2], and is recognised as the organism most closely resembling the early fish-like vertebrate progenitors[3,4]. Recently, however, Manwell[5] has provided evidence that, with respect to enzyme variability, amphioxus resembles invertebrates rather than vertebrates. We report here that, in terms of gross sequence characteristics of DNA, amphioxus and two other protochordates, *Ascidia mentula* and *Ciona intestinalis*, more closely resemble the DNAs of the invertebrates, echinodermata and brachiopoda, rather than the DNA of higher vertebrates (craniata).

Characterisation of the DNA was by nearest-neighbour two-base sequence (doublet frequency) analysis and subsequent determination of the patterns of deviation from random expectation of the doublet frequencies (general designs)[6–8]. The technique of doublet frequency analysis, originally developed by Kornberg and his colleagues[9–11], measures the frequencies of occurrence of the sixteen possible doublet sequences in DNA. Normalisation of the observed doublet frequencies to give the general design yields a characteristic pattern which has six degrees of freedom for a double-stranded DNA. Expressing doublet frequencies as patterns of deviation from random expectation removes the components of variation in frequency which are a consequence of base composition alone, leaving an underlying pattern of sequence-dependent characteristics which are independent of G+C content. This is a very stable characteristic of DNA (refs 7 and 12), and similarity in general designs in DNAs is considered to reflect past response to similar or identical evolutionary selection pressures (and is thus a measure of evolutionary relatedness). When general designs were calculated from the results of Swartz *et al.*[10] for three echinoderms (2 echinoidea and 1 asteroidea) and six vertebrates (1 fish, 1 bird and 4 mammals), the echinoderm and vertebrate DNAs fell into two readily distinguishable groups while the DNAs within each group were closely similar[12].

Table 1 gives the doublet frequencies and base compositions of three protochordate DNAs from amphioxus (*Branchiostoma lanceolatum*), *Ciona intestinalis* and *Ascidia mentula*, two brachiopod DNAs from *Crania anomala* and *Terebratulina* sp. and two echinoderm DNAs from *Antedon bifida* (Crinoidea) and *Thyone fusus* (Holothuroidea). The lower craniates are represented by the DNAs of two fish; the primitive cartilagenous dogfish (chondrichthyes) and the higher bony fish, coelacanth (osteichthyes; crossopterygii). For comparison, Table 1 includes the doublet frequencies determined by Swartz *et al.*[10] for the echinoderm, *Echinus esculenta* (Echinoidea) and the higher fish, salmon (Osteichthyes; Actinopterygii), the most primitive vertebrate previously analysed. Also shown are the G+C contents gained from doublet frequency data and, where available, from buoyant density determinations. The observation that G+C contents calculated from doublet frequency data are generally slightly lower than those given by buoyant densities has already been noted[8,13]. It is relevant that none of the DNAs was found to contain any significant proportions of density satellite DNAs. Even on close examination of the doublet frequency data of Table 1, it is difficult to come to any firm conclusions with regard to comparisons of the invertebrate and craniate DNAs. However, when the general designs (that is, functions of sequence characteristics independent of total base composition) are compared (Fig. 1), those of the protochordates are found to be very similar, not only to each other, but also to the general designs of the echinoderms and brachiopods. Like these invertebrates, the protochordates differ from the lower craniates with particular reference to the relative frequencies of the doublets AG-CT and CG.

Evaluation of the differences seen in the histogram presentation of the general designs was achieved by cluster analysis using the weighted average linkage method[14] with Euclidean squared distance. This was done for the general designs shown in Fig. 1 together with those of the DNAs of the higher vertebrates: guinea pig (Mammalia), chicken (Aves) and bullfrog (Amphibia) and the echinoderms *Paracentrotus lividus* (Echinoidea) and *Asterais rubens* (Asteroidea). The resulting dendrogram (Fig. 2) clearly shows that the craniate and protochordate DNAs fall into separate groups. The protochordates are indistinguishable from the invertebrates by this method. The small differences within the protochordate–invertebrate group are not much greater than the experimental error inherent in doublet analysis[13], and differences in undetected satellite populations could have contributed here. The larger differences seen within the vertebrates are undoubtedly real with the design remaining stable from mammalia to amphibia, but with differences becoming pronounced at, and within, the fish. Some of these differences may be due to satellite DNAs, but in the dogfish the general design for slow renaturing nuclear DNA is identical to that of total nuclear DNA (unpublished results).

Table 1

	Brachiopods		Echinoderms			Protochordates			Craniates		
	Crania	*Terebratulina*	*Echinus*	*Antedon*	*Thyone*	*Ascidia*	*Ciona*	*Branchiostoma* (Amphioxus)	Dogfish	Coelacanth	Salmon
AT	104	105	91	119	87	82	94	83	70	71	73
TA	90	92	75	109	68	70	77	71	53	62	68
AA TT	121 124	119 128	100 116	119 119	81 90	96 105	82 92	86 99	73 69	103 109	74 83
GT AC	55 56	56 55	53 62	58 52	60 57	62 58	61 55	65 59	61 59	55 48	62 62
TG CA	66 65	67 65	69 76	68 62	72 70	75 72	72 68	77 71	82 76	73 66	75 77
GA TC	53 53	51 52	49 64	51 47	61 62	52 56	56 50	58 60	74 68	52 56	57 67
AG CT	49 50	48 49	47 64	50 47	55 55	54 57	52 54	58 60	75 72	61 65	69 75
GG CC	34 32	32 32	31 46	29 24	55 50	44 44	54 49	44 43	59 54	58 59	49 52
GC	27	27	35	28	41	45	39	40	40	45	40
CG	20	20	22	18	35	30	32	28	18	18	17
a	32.9	32.8	30.0	34.2	28.1	29.0	28.5	28.5	27.6	28.3	27.6
t	33.3	33.9	32.4	34.2	29.3	30.5	30.2	30.6	27.1	30.0	29.3
g	17.0	16.7	16.8	16.6	21.7	20.3	21.0	20.7	23.4	21.0	21.0
c	16.7	16.7	20.8	15.1	21.0	20.3	20.3	20.1	21.9	20.8	22.1
%G+C	33.7	33.4	37.6	31.6	42.7	40.5	41.3	40.8	45.3	41.7	43.1
ρ	1.696	1.695	—	*	1.704	1.701	1.703	1.702	1.704	*	—
%G+C from ρ	36.4	35.9	—	*	44.8	41.9	43.4	42.6	44.9	*	—

'Total DNA' preparations were purified from tissue homogenates (1 g per 5 ml 0.15 M NaCl, 0.1 M EDTA; *p*H 8.0) by treatment with SDS and Pronase[15], followed by two cycles of banding at equilibrium in neutral CsCl buoyant density gradients[16]. Density satellite DNAs were not detected in these preparative gradients and each DNA appeared as a single band in analytical neutral density gradients. Doublet frequencies of the purified DNAs were determined as described by Josse *et al.*[9] with minor modifications[17,18]. The percentage base compositions and G+C contents were determined from doublet frequency data. Also given are the G+C contents derived from the buoyant density measurements shown. Exceptions are *Antedon** and coelacanth* where the molecular weights of the isolated DNAs were too low for accurate evaluation of the peaks. Doublet frequencies are expressed in doublets per 1,000 and doublets shown in pairs are the dependent doublets[7,8], that is, those doublets which are complementary in the anti-parallel strands and are theoretically present with equal frequencies in double-stranded DNA. The observed values therefore give no measure of the frequencies on any one strand of the DNA. Doublets shown singly are the independent doublets, each being its own anti-parallel complement with the observed values being true for either strand of the DNA.

It can therefore be concluded that, in terms of gross sequence characteristics, protochordate DNA more closely resembles the DNAs of the invertebrates, echinodermata and brachiopoda, than the DNAs of the craniates. Other invertebrate DNAs which have so far been examined (for example, arthropod DNAs; G.J.R. and J.H.S.-S., in preparation) are even more dissimilar to the craniate pattern than the protochordate, echinoderm and brachiopod DNAs.

On the basis of taxonomic criteria[1,2], amphioxus has become accepted as the 'prototype' chordate closely related to the ancestors of the earliest craniates[3,4]. However, using the criterion of enzyme polymorphism, Manwell has now suggested that the protochordate amphioxus is much more like invertebrates than vertebrates. Our findings, based on a very stable general characteristic of the total genome, not only provide strong support for Manwell's observation, but also show that the protochordates as a group relate much more closely to the higher invertebrates than to the craniates.

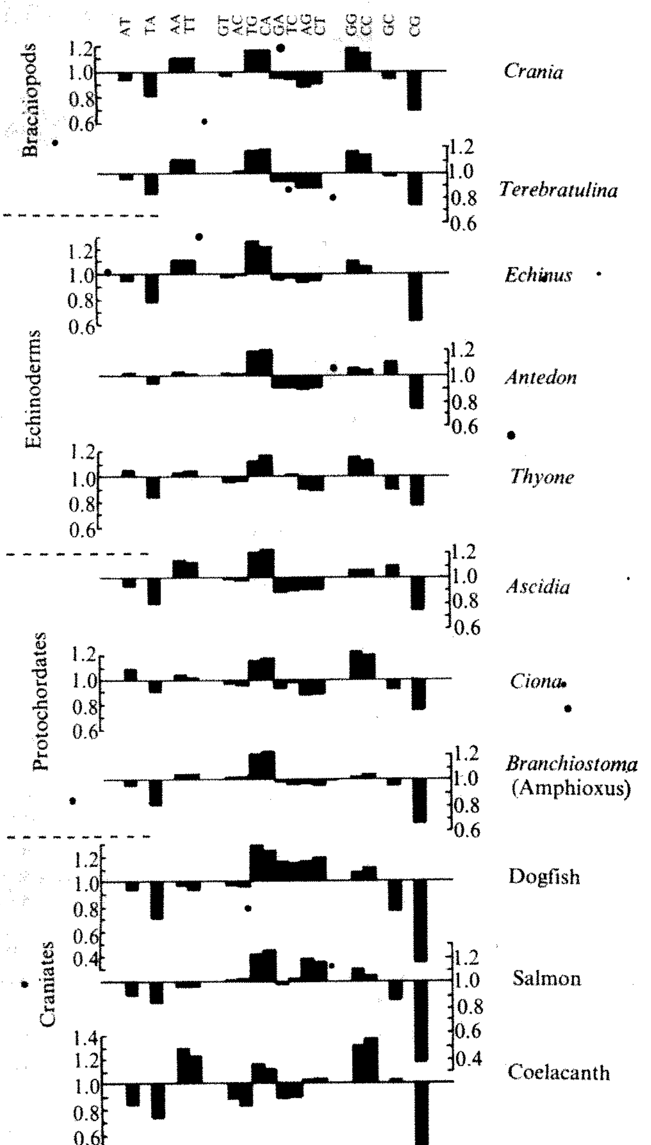

Fig. 1 Deviations from random expectation. Patterns of deviation from random expectation were determined from the doublet frequencies and base compositions shown in Table 1 by calculating the ratios of the observed doublet frequencies to the expected random frequencies[8]. The expected random frequency of a particular doublet is the product of the frequencies of the bases comprising that doublet. Both doublet frequencies and the base frequencies were expressed as fractions of unity in order to determine the ratios. A doublet with a random frequency would therefore give a ratio of one and the results are expressed in histogram form as deviations from unity. The doublet CG in salmon, for example, has a frequency of 17 doublets per 1,000 (0.017). The two bases, C and G, occur with frequencies of 22.1% (0.221) and 21.0% (0.220) respectively. The expected random frequency of a two-base sequence containing C and G is therefore $0.221 \times 0.220 = 0.046$, and the ratio of the observed frequency of CG to the expected random frequency is therefore $0.017/0.046 = 0.37$, that is, salmon DNA has only 0.37 times the expected amount of CG.

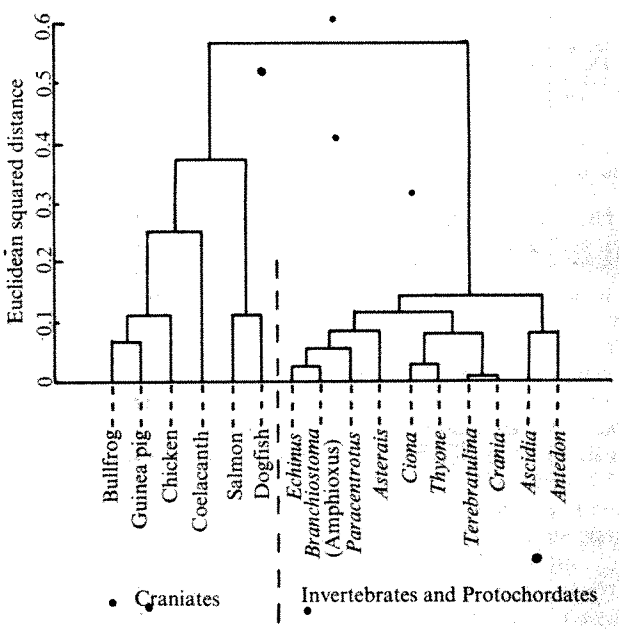

Fig. 2 Cluster analysis of the general designs of vertebrate, protochordate, echinoderm and brachiopod DNAs. The weighted average linkage method was used with Euclidean squared distance (that is, the sum of the squares of differences of ratios of observed to random frequencies between two DNAs) as the measure of distance[14]. The general design values for chicken, salmon, *Echinus*, *Paracentrotus* and *Asterias* DNAs were calculated from the results of Swartz *et al.*[10]. The values for bullfrog DNA are from McGeoch[10] and those for guinea pig DNA are from Russell *et al.*[13].

Diversification of general designs in other invertebrate phyla are being further investigated for comparison with existing phylogeny. There clearly remains a wide gap between the protochordates (acrania) and craniates, and analyses of other primitive chordates, such as cyclostomata (lamprey), are obviously needed.

The doublet frequencies for *Echinus esculenta* and salmon are from Swartz *et al.*[10]. We thank Dr R. H. Harris, Liaison Officer of the Coelacanth Committee of the Royal Society, for providing the coelacanth tissue.

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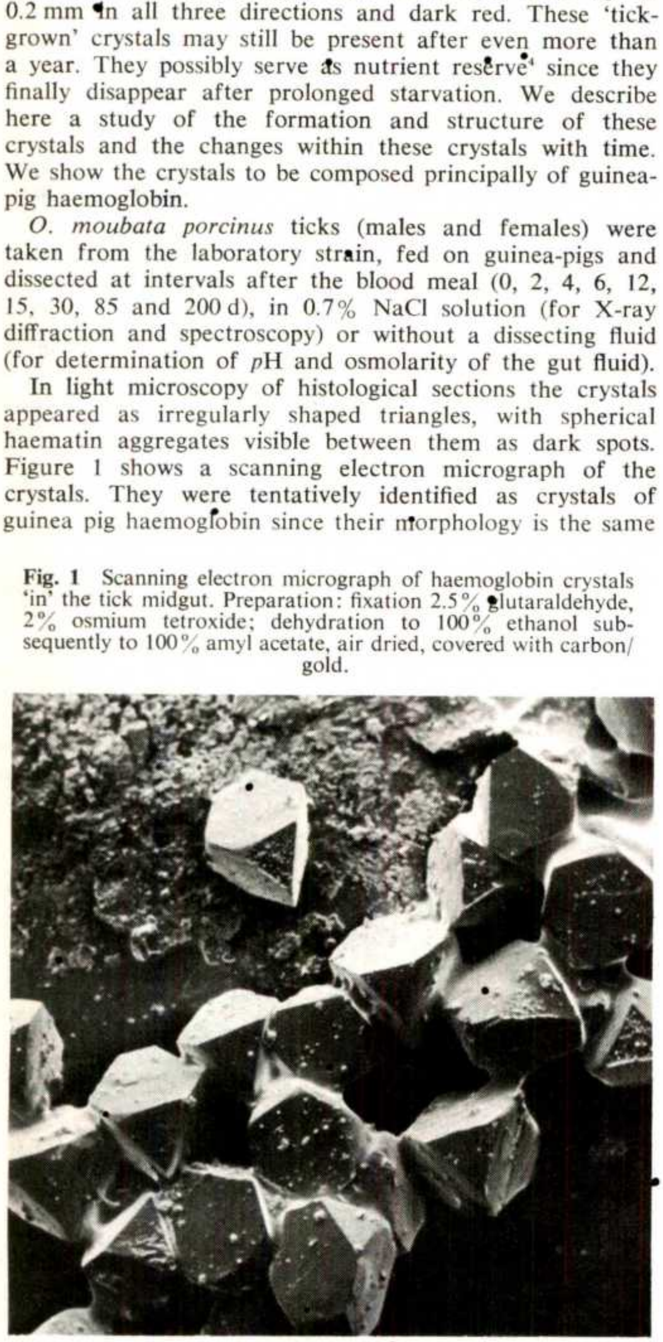
Haemoglobin crystals in the midgut of the tick *Ornithodorus moubata* Murray

Ticks of the *Ornithodorus moubata* Murray (Ixodoidea, Argasidae) complex, distributed through East Africa, are known as vectors of African relapsing fever[1]. A laboratory strain of *O. moubata porcinus* (originally from Tanzania and bred according to the method of Geigy and Herbig[1]) uses the guinea-pig (*Cavia porcellus* L.) as experimental host. Guinea pig blood crystallises more easily than that of other hosts[2] in the gut of blood-sucking arthropods[3]. In *O. moubata* crystals appear 5–10 d after the blood meal, first small (0.015–0.05 mm) and clear red, later up to 0.2 mm in all three directions and dark red. These 'tick-grown' crystals may still be present after even more than a year. They possibly serve as nutrient reserve[4] since they finally disappear after prolonged starvation. We describe here a study of the formation and structure of these crystals and the changes within these crystals with time. We show the crystals to be composed principally of guinea-pig haemoglobin.

O. moubata porcinus ticks (males and females) were taken from the laboratory strain, fed on guinea-pigs and dissected at intervals after the blood meal (0, 2, 4, 6, 12, 15, 30, 85 and 200 d), in 0.7% NaCl solution (for X-ray diffraction and spectroscopy) or without a dissecting fluid (for determination of *p*H and osmolarity of the gut fluid).

In light microscopy of histological sections the crystals appeared as irregularly shaped triangles, with spherical haematin aggregates visible between them as dark spots. Figure 1 shows a scanning electron micrograph of the crystals. They were tentatively identified as crystals of guinea pig haemoglobin since their morphology is the same as described for those crystals[5]. After soaking in a standard 3 M $(NH_4)_2SO_4$ solution buffered with 0.1 M potassium phosphate at *p*H 6.7 (*p*H measured after 10-fold dilution) the crystals were further characterised by taking 16° precession photographs of single crystals.

Fig. 1 Scanning electron micrograph of haemoglobin crystals 'in' the tick midgut. Preparation: fixation 2.5% glutaraldehyde, 2% osmium tetroxide; dehydration to 100% ethanol subsequently to 100% amyl acetate, air dried, covered with carbon/gold.



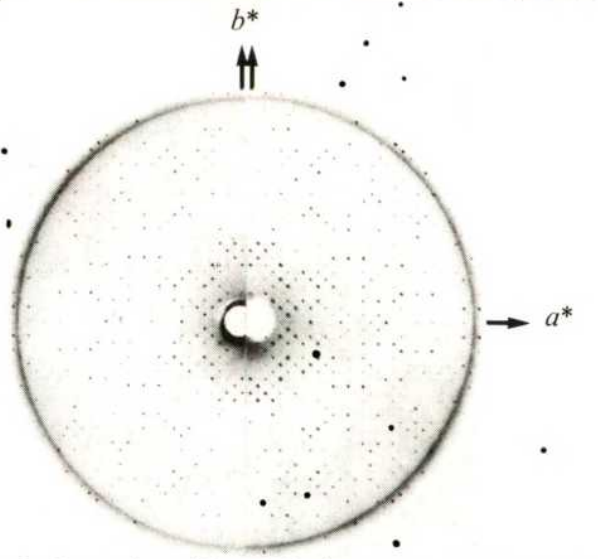


Fig. 2 Comparison of X-ray diffraction patterns of 'tick-grown' (left half) and '*in vitro*-grown' (right half) crystals. 16° Precession photographs (2.8 Å resolution) of the *hk*0-zone, CuKα radiation, graphite monochromator. Arrows indicate reciprocal axes.

Haemoglobin from guinea pigs of the same strain was purified on DEAE–Sephadex[6]. Single crystals of the major haemoglobin component (~80% of total haemoglobin) were grown from 1 and 4% solutions, using the Zeppezauer technique[7], from either 3 M $(NH_4)_2SO_4$ solutions, buffered with 0.1 M potassium phosphate at *p*H 6–7, or from 3 M potassium phosphate solutions in the same *p*H range. These '*in vitro*-grown' crystals were soaked in the same standard solution as the 'tick-grown' crystals and 16° precession photographs were taken for comparison.

The '*in vitro*-' and 'tick-grown' crystals are both orthorhombic and, since extinctions are observed for $h+k$ odd for general *hkl*- and for *l* odd for 00*l*-reflections, belong to space group $C222_1$. The cell dimensions are $a=84.5\pm0.05$ Å, $b=89.9\pm0.1$ Å (ref. 5), $c=83.1\pm0.05$ Å and they are the same for the two types of crystals. On the basis of the observed range of V_M values for a variety of protein crystals[8], there can only be four haemoglobin molecules (molecular weight 64,500 each) per unit cell. The V_M value[8] is then calculated to be 2.45 Å^3 per dalton. The alternative of eight tetramers per unit cell would lead to the unrealistically low V_M value of 1.22 Å^3 per dalton (<0% crystal solvent). Since space group $C222_1$ has eight general positions[9] the haemoglobin tetramers must lie on the crystallographic axes parallel to either the *a* or the *b* axis as has been found for reduced horse haemoglobin[10]. The intensity patterns to 2.8 Å resolution are identical on visual inspection for '*in vitro*-' and 'tick-grown' crystals (Fig. 2) and we therefore conclude that the 'tick-grown' crystals are in fact composed of guinea pig haemoglobin.

In order to exclude even small changes, such as scission of the chain at one or a few discrete points or limited degradation from the termini, which might explain why guinea-pig haemoglobin crystallises so readily in the midgut of *O. moubata*, we analysed the 'tick-grown' crystalline material by sodium dodecyl sulphate–polyacrylamide gel electrophoresis[11] (12.5% gels, cross linked 1: 15). The sharpness of the protein bands was dramatically increased by using sodium phosphate instead of Tris-HCl buffer. 'Tick-grown' crystals were thoroughly washed with standard 3 M $(NH_4)_2SO_4$ solution, to remove any non-crystalline material,

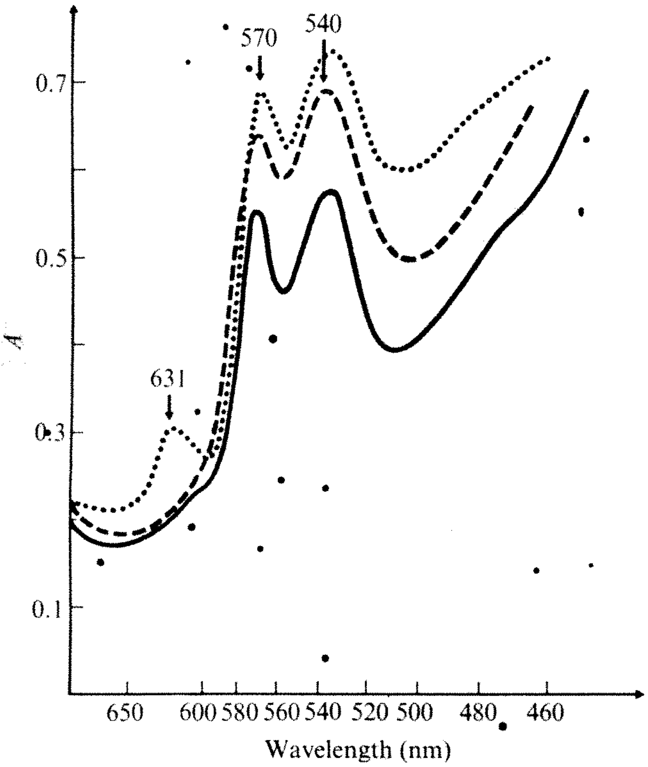


Fig. 3 Spectra from single 'tick-grown' crystals. Crystal from gut of male tick 6 d after blood meal on guinea pig (——). Crystal from gut of male tick 30 d after meal (· · · ·). Same crystal (30 d after meal) after addition of KCN to 0.01 M (– – –). The methaemoglobin peak at 630 nm disappeared after addition of KCN. Spectra were taken on the universal microspectrophotometer of the Wissenschaftlicher Dienst/Kriminalpolizei Zuerich. The wavelength scale is given by the dispersion of the quartz monochromator (M4QIII). Solvent was 3 M ammonium sulphate, 0.1 M potassium phosphate buffered at *p*H 6.7, saturated with CO. Specific absorption maxima occur for CO-haemoglobin at 540 and 570 nm, for aquomethaemoglobin at 631 nm, and for cyanmethaemoglobin at 540 nm. Conclusion—guinea pig haemoglobin crystallises in tick gut in oxy-form but transforms *in vivo* into methaemoglobin crystals with time.

and subsequently dissolved in sodium phosphate buffer. The main haemoglobin component of guinea pig blood served as a reference. In these conditons are α and β chains of guinea pig haemoglobin are clearly separated in the crystalline material and the reference haemoglobin. The R_F values are 0.452 and 0.502 for the two bands of the crystalline material and 0.448 and 0.491 for the reference haemoglobin. Calibration with lysozyme, human globin A and myoglobin and the difference in relative mobility between α and β chains of guinea pig haemoglobin yielded a difference in molecular weight of about 650. In human haemoglobin[12] the difference between α and β chains is 741 daltons. On ageing the guinea pig haemoglobin preparation gave broader electrophoretic bands as observed for carp haemoglobin[13]. The combined probe of 'tick-grown' crystalline material and fresh haemoglobin showed only two bands demonstrating the lack of significant fragmentation or extensive degradation from the termini. Removal of only one or two residues cannot be entirely excluded.

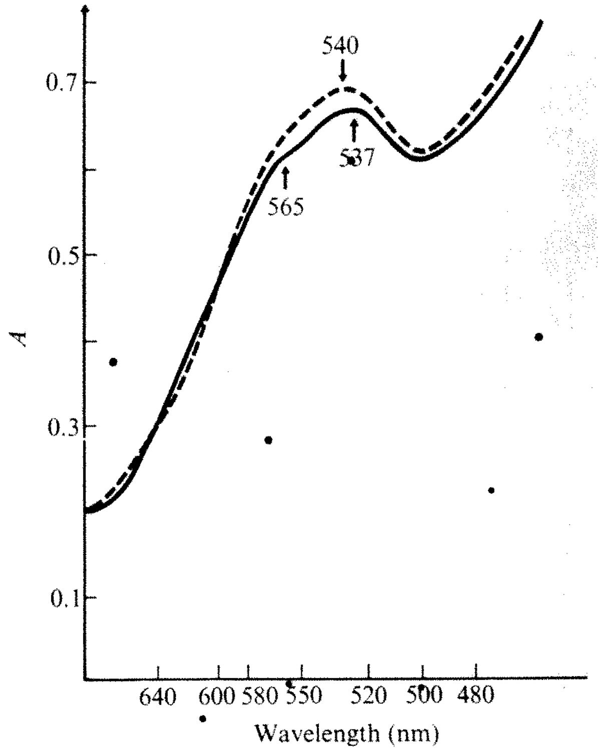
A still more sensitive test for the conformation of haemoglobin and the oxidation state of its iron in 'tick-grown' crystals is visible absorption spectroscopy on single crystals and comparison with absorption spectra of haemoglobin in solution[14]. Conversions of crystalline haemoglobin were followed by recording spectra from single crystals of different age (Fig. 3) obtained at different time intervals after the blood meal. Immediately after dissections the contents of the midguts were brought under a CO atmosphere to freeze the momentary equilibrium between Fe^{2+} and Fe^{3+}. From Fig. 3 we conclude that haemoglobin crystallised in the tick midgut in the oxy-form but with time it transformed into methaemoglobin. This, however, is not the end state of the crystalline haemoglobin since the absorption spectra of single crystals from a tick dissected 15 months after its last blood meal show a typical haemichrome absorption spectrum with a single absorption maximum at 537 nm and a shoulder at 565 nm (Fig. 4, solid line). On soaking in a cyanide-containing solution (standard 3 M $(NH_4)_2SO_4$, 0.01 M KCN) the absorption maximum at 537 nm shifts to 540 nm and the shoulder at 565 nm almost disappears (Fig. 4, dashed line). Thus the haemichrome is not yet entirely in its irreversible state since, on KCN addition, it can be partially converted to cyanmethaemoglobin. Apparently with time methaemoglobin in the 'tick-grown' crystals transforms *in vivo* into haemichrome. The transformation to haemichrome is not associated with cracking of the crystals since, in X-ray diffraction experiments, the haemichrome-containing crystals still diffract well and behave as single crystals.

From the above it is clear that partial degradation or conformational changes are not the cause of the crystallisation of guinea pig haemoglobin in the midgut of *O. moubata*. Other factors which could effect crystallisation are changes in *p*H, osmolarity, or concentration of the blood meal.

The osmolarity of midgut fluid and of guinea-pig blood determined with a Knauer Halbmikro-osmometer was 300 milli-equivalent per kg. Changes in the osmolarity are therefore not responsible for the crystallisation.

Directly after a meal the midgut fluid *p*H is 7.5. It drops later to 6.5. This decrease initiates earlier in males (from the 6th day after the meal) than in females (from the 12th day). It is not clear whether this *p*H drop triggers the crystallisation. Other rodent haemoglobins are relatively insoluble in acid environment[15]. The decrease in *p*H could

Fig. 4 Spectra from single 'tick-grown' crystal. Crystal showing spectrum of haemichrome (——). Same crystal, spectrum partially converted to cyanmethaemoglobin after addition of KCN to 0.01 M (– – –). Conditions as in Fig. 3. Conclusion—methaemoglobin crystals *in vivo* undergo transformation into haemichrome crystals with time. This is not associated with cracking of the crystals.



also be caused by loss of haemoglobin from solution on crystallisation. *In vitro* experiments show that a decrease in *p*H results in an increased solubility of guinea pig haemoglobin. So it seems more likely that the decrease in *p*H is a result of the crystallisation (and of cellular disintegration processes) rather than its cause.

Concentration of the meal takes place by elimination of water through the coxal organ[16]. The concentration factor of guinea pig haemoglobin in the midgut fluid compared with haemoglobin in guinea pig blood was determined by Drabkin's method[14] on 10 μl of fluid or blood. For ticks, dissected 2 h 45 min after their blood meal, a concentration factor of about 1.9 was found which compares well with the value of 1.72 found by Kaufman[17] 2 h after completion of the blood meal. Crystallisation in the midgut starts only after several days, thus the initial, about 2-fold, concentration of the meal is not directly responsible for it. It is, however, possible that concentration of the meal, after the initial steep phase, goes on at a reduced rate and thus eventually triggers crystallisation.

Guinea pig haemoglobin crystallises in the midgut of the tick *O. moubata* as oxyhaemoglobin and, with time, transforms to methaemoglobin and then to haemichrome without disruption of the crystals. Partial haemoglobin degradation or changes in *p*H or osmolarity of the midgut fluid are not responsible for crystallisation. Continued concentration of the blood meal may have a role.

In tick biology, the crystals seem to have an important but not essential role in the preservation of the nutrient reserves since Argasids fed with human blood (which does not crystallise[2]) may also starve for very prolonged periods. It is not known how the nutrient stored in the crystal is made available for intracellular digestion by the starving tick. Distortion of the equilibrium between solid state (crystals) and liquid state (midgut fluid) by the digestive activity of the midgut cells, however, could lead to a gradual solvation of the crystals.

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Effect of a tumour promoter on myogenesis

EVIDENCE that viruses and chemical carcinogens alter the ongoing synthetic programme of cells has been presented[1,2]. Chondroblasts and melanoblasts transformed with a temperature-sensitive Rous sarcoma virus cease synthesising the chondroblast-unique sulphated proteoglycan and melanin, respectively[3]. Shifted to non-permissive temperature, these cells re-initiate the synthesis of the chondroblast-unique sulphated proteoglycan and melanin. Similar results have been obtained using chick myogenic cells[4,5]. Normal presumptive myoblasts undergo a limited number of replications, yielding postmitotic, mononucleated myoblasts. Postmitotic myoblasts initiate the synthesis of muscle-specific myosin heavy and light chains and fuse into myotubes[6,7]. In contrast, ts-viral transformed myogenic cells continue to replicate, do not initiate the synthesis of muscle-specific myosins and do not fuse. 24–48 h after shifting to non-permissive temperature, many transformed myogenic cells withdraw irreversibly from the cell cycle, initiate the synthesis of muscle-specific (unpublished data) myosins and fuse. Apparently when the transformation factor is expressed the cells do not withdraw from the cell cycle, and as a consequence of being forced to cycle, do not terminally differentiate. To probe the mechanisms

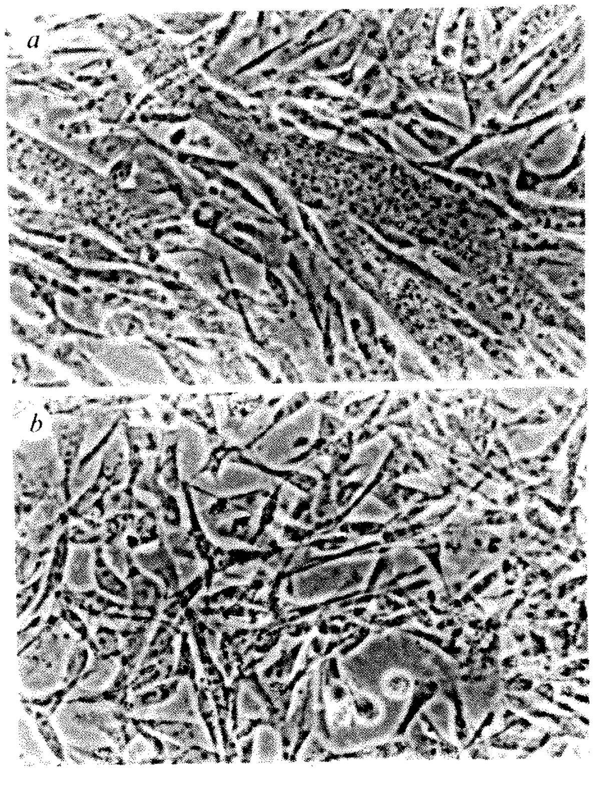

Fig. 1 Phase micrographs of living 4-d primary muscle cultures grown in *a*, normal medium and *b*, normal medium plus PMA. The myotubes may achieve lengths of over 1 mm, may contain hundreds of postmitotic nuclei, and contract spontaneously. The replicating mononucleated cells in both cultures are in various compartments of the myogenic and fibrogenic lineages, and cytologically the cells in these lineages cannot be distinguished. The vast majority of cells in PMA-treated cultures are small bipolar cells. Under higher magnification occasional bi- and trinucleated cells are observed in the PMA-treated cultures; the frequency of such cells is less than 1 per 10^3 mononucleated cells. The multilayered arrangement and overlapping of cell processes demonstrates the absence of density-inhibited replication and migration in the PMA-treated cultures.

that might be common to the generation of terminally differentiated cells from normal precursor cells, and to the generation of malignant cells from normal precursor cells, we studied the effects of phorbol-12-myristate-13-acetate (PMA) on myogenesis. Of the purified phorbol esters, PMA is the most potent as a tumour-promoting agent and as a mitogen[8-12]. We report here that PMA mimics some of the effects of the Rous sarcoma tumour agent on myogenesis.

Primary myogenic cultures were prepared from muscles of 11-d-old chick embryos[13]. PMA (5×10^{-8} M) was added at various times. Muscle-specific myosin heavy chains were assayed, using antibodies specific for muscle heavy chains. Some properties of these myosin antibodies are detailed in Fig. 2 and refs 6 and 7. The actomyosin was extracted from cells by the method of Adelstein *et al.*[14]. Muscle-specific myosin light chains were determined by running partially purified actomyosin extracts in SDS–polyacrylamide gels[7,15]. Postmitotic, mononucleated myoblasts were collected by treating primary cultures with Cytochalsin-B[6,16] or EGTA plus cytosine arabinoside (Ara-C)[7,17].

A micrograph of a control 4-d culture is shown in Fig. 1*a*. Over 50% of all nuclei were in myotubes. The myosin heavy chains from these cultures formed a single precipitin band with antisera against muscle myosin (Fig. 2). Actomyosin extracts in SDS–polyacrylamide gels exihibited muscle-specific, fast myosin light chains, LC_1 and LC_2, with molecular weights of 25,000 and 18,000 (data not shown)[7,15].

Figure 1*b* is a micrograph of a culture reared continuously in PMA for 4 d and clearly shows the absence of myotubes. Myosins from these cells did not form precipitin bands with antibody against muscle-specific myosin (Fig. 2). Furthermore, the light chains from these cells co-migrated with the light chains from presumptive myoblasts or fibroblasts and have MWs of 20,000 and 16,000.

When myogenic cells grown in PMA for 6 d were shifted to normal medium, many withdrew from the cell cycle and terminally differentiated within 24–48 h. In another series cells were subcultured in PMA for 1 or 2 passages. During each passage the number of cells increased over sevenfold (Table 1). The cells did not fuse during these subcultures. At the end of the first or second subculture the cells were shifted to normal medium. Within 24–48 h many cells withdrew from the cell cycle, fused and terminally differentiated. The ratio of myotube nuclei/total nuclei in these 'reversed' cultures equalled that of primary cultures. These findings, and additional autoradiographic data are shown in Table 1. The mitogenic effect of PMA (Table 2) preceded fusion, which in these cultures begins on day 3 and peaks on day 4. This type of mitogenic effect with PMA is also observed with ts-transformed myogenic cells and with cells that have incorporated BudR into their DNA[13].

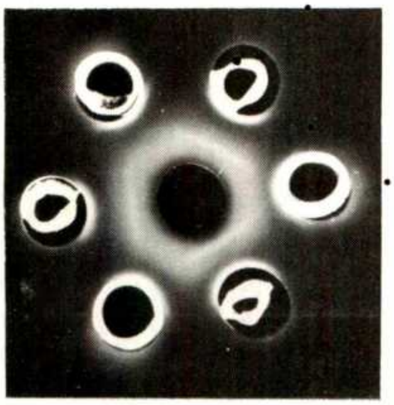

Fig. 2 Control and PMA-treated cultures were extracted for actomyosin and diffused against antibody to adult chicken skeletal mysoin. In the experiment shown, this particular myosin antibody in Ouchterlony tests did not form precipitin bands against C protein[26]. Similar antibodies did not precipitate myosin from chicken cells (replicating presumptive myoblasts, fibroblasts, mesenchyme, smooth muscle, embryonic nerve cells or Rous-transformed myogenic cells grown at permissive temperature) or human blood platelets or mouse Lcells. This antibody did form precipitin bands with acto-myosin extracted from (1) postmitotic precipitin bands with acto-myosin extracted from (1) postmitotic, mononucleated myoblasts, (2) normal myotubes and (3) myotubes that formed when Rous sarcoma ts-transformed myogenic cells were reared at non-permissive temperature (refs. 6, 7, 16, 18 and unpublished results). Actomyosin solutions, antibody solutions and gels were equilibrated with 0.4 M KCl 0.03 M phosphate buffer, *p*H 7.3: 40 µl of solution were placed in each well and allowed to diffuse at 4 °C for 48 h. The gels were washed on phosphate buffer for 24 h, stained with thiazine in H_2O and destained with 1% acetic acid. Wells are numbered clockwise and the upper right-hand well is no. 1. The centre well contains antibody to adult skeletal chick myosin. Wells 1, 3 and 5 contain 4 mg ml^{-1} actomyosin from control cultures. Wells 2, 4 and 6 contain 5 mg ml^{-1} actomyosin from PMA-treated primary cultures. In other experiments, similar results were obtained when the protein concentration of actomyosin from cells reared in PMA was four times that of control cultures. Lastly, fluorescein-labelled antimysoin experiments performed as described in refs 7, 25 yielded results consistent with the agar diffusion tests. For this photograph the stained gel served as a negative.

The following experiments demonstrate that the mitogenic target of PMA was the replicating presumptive myoblasts and fibroblasts, and that PMA was not a mitogen for nuclei in the definitive, mononucleated myoblasts or the nuclei within myotubes. Mononucleated myoblasts synthesising muscle-specific myosins were collected by treating primary cultures with cytochalasin-B or EGTA

Table 1 Autoradiographic analysis of standard, PMA-treated and PMA-reversed myogenic cultures

	% of all labelled nuclei per dish		% of all unlabelled nuclei per dish	
	Nuclei in mononucleated cells	Postmitotic nuclei in myotubes	Nuclei in mononucleated cells	Postmitotic nuclei in myotubes
Standard 4-d	26	0.2*	28	46
primary cultures	20	< 0.1*	25	55
4-d PMA-treated	51	0	49	0
primary culture	46	0	52	0
PMA-treated primary culture subcultured into normal medium	28	1*	20	51
PMA-treated	52	0	48	0
primary culture subcultured into PMA-medium	59	0	41	0

Late 3-d cultures were exposed to ^{3}H-TdR for 10 h, processed for autoradiography, and cells counted as described[13]. Initial inoculum 0.5×10^6 nuclei per 35-mm Petri dish. In standard 4-d cultures there were 3×10^6 to 4×10^6 nuclei per dish, of which roughly 50% were postmitotic and in myotubes. In PMA-treated primaries there were 4×10^6 to 5×10^6 nuclei per dish, virtually all mononucleated. Secondary cultures of PMA-treated cells if again grown in PMA achieved densities of up to 6×10^6 nuclei per dish after 4 d. If, however, PMA-treated primaries were grown as secondaries in normal medium, they again achieved densities of 3×10^6 to 4×10^6 nuclei per dish, of which 50% were postmitotic and in myotubes. Additional experiments are required to determine what percentage of unlabelled mononucleated cells in both types of culture were in fact (1) definitive postmitotic myoblasts, and (2) myogenic and fibrogenic cells that simply did not enter S during the period in ^{3}H-TdR. It should be stressed that normal and PMA-treated cultures did not cease replicating when confluent, but multilayered.

*The small percentage of labelled nuclei in myotubes were due to the fusion of labelled daughter cells whose mothers passed through at least the later part of S and then divided during the 10 h the cells were in ^{3}H-TdR.

Table 2 DNA determination of controls and PMA-treated cultures

Type of culture	DNA content (μg per dish) at various times 48 h	72 h	96 h	120 h
Control primary	52	93	144	144
PMA-treated primary	70	147	235	292

Each value (mean of two separate experiments performed at different times) represents the amount of DNA (in μg) per 100 mm Petri dishes. Initial inoculum 0.5×10^6 cells per ml, 8 ml per dish. DNA was determined by the method of Burton[24]. The values for the 120-h cultures are difficult to interpret. In both types of cultures cells are lost into the medium. In the controls, entire multinucleated myotubes often degenerate, whereas in the PMA-treated cultures many mononucleated cells are displaced from the substrate and die.

plus Ara-C[6,7,16]. These definitive myoblasts were subcultured into medium with PMA and ^{3}H-TdR or with ^{3}H-TdR alone, and both series autoradiographed. Less than 2% of the cells incorporated the label after 36 h in both series. In subcultures prepared from untreated primaries, over 70% of the cells were labelled after 36 h. Not a single nucleus within a myotube was labelled when PMA plus ^{3}H-TdR was added after myotubes had formed.

Fusion is the end step of several distinct events. First, the generation by way of cell divisions of postmitotic, fusible-competent myoblasts[18]. Second, the mutual recognition, lateral alignment and adhesion of these postmitotic myoblasts[19–21]. And third, the physical event of fusion itself. Fusion, as determined with living cells under the phase microscope, requires no more than 30–60 min[22,23]. The following demonstrates that, in addition to acting as a mitogen, PMA blocks the physical event of fusion. Fusible-competent, postmitotic myoblasts were collected from cultures treated with cytochalsin-B or EGTA plus Ara-C. When these myoblasts were subcultured into normal medium, over 60% fused within 15–20 h. When these definitive myoblasts were subcultured into medium with PMA, fusion was blocked, although as mononucleated cells they continued to synthesise the muscle-specific myosin heavy and light chains.

With respect to promoting cell replication, PMA-treated myogenic cells mimic the behaviour of myogenic cells transformed with ts-Rous sarcoma virus, and of myogenic cells that incorporated BudR into their DNA. Possibly PMA, the ts-virus and BudR all exert their common effect on myogenesis by forcing cells in the penultimate compartment of the myogenic lineage to replicate. In addition, however, PMA, but not the virus or BudR, blocks the event of fusion. It may be that PMA acts directly on the cell surface of replicating presumptive myoblasts and postmitotic myoblasts. The former are inhibited from pulling out of the cell cycle, the latter from fusing. In any case, the final effect of such dissimilar agents as Rous virus transforming factor, BudR, and PMA seems to be to force presumptive myoblasts to undergo proliferative cell cycles which in turn precludes the flipping of the 'master-switch' which permits activation of the synthetic program of the post-mitotic mononucleated myoblast[5,7,20,25].

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Human LD antigens are present on xenogeneic cells

THE mixed leukocyte culture (MLC) reaction is largely a measure of specific responsiveness of thymus-derived lymphocytes to foreign allogeneic and xenogeneic major histocompatibility complex (MHC) controlled LD antigens[1]. In view of the remarkable primary structure homologies of MHC-controlled SD antigens between humans and mice[2], we have investigated whether MHC LD antigens have also been conserved in evolution by studying the extent of LD sharing between different species. Using the primed lymphocyte typing (PLT) test, we have found extensive sharing of LD antigens between humans and monkeys, and less, but still significant, sharing between humans and dogs and humans and cattle. In no case was there evidence that mouse LD antigens were shared with those of humans.

Human lymphocytes cultured in primary allogeneic MLC proliferate extensively and revert to small lymphocytes by day 10. If then restimulated with the original sensitising cell these primed lymphocytes (PLT cells) give a much more rapid proliferative response, which can be assayed by ^{3}H-TdR incorporation as early as 24 h. This secondary, or typing, response is specific for HLA LD determinants and is also elicited by random restimulating cells (test cells) that share LD determinants with the original sensitising cell[3]. PLT cells can also be generated in xenogeneic combinations and appear specific for the LD antigens of the sensitising species[4].

If individual human cells are sensitised to a pool of 20 allogeneic stimulating cells, the resulting PLT cells (pool-PLT cells) seem sensitised to essentially all human LD determinants, as virtually any human test cell used for restimulation elicits a strong secondary response[5]. Test cells that elicit a poor secondary response from a pool-PLT cell must either share LD determinants with the responding cell or carry LD determinants that are not represented in the sensitising cell pool. The primary MLCs between test and responding cells distinguish between these two possibilities. The magnitude of the secondary response correlates very well with that of the primary MLC ($r=0.89$) (Fig. 1). This indicates that human test cells that fail to restimulate a pool-PLT cell fail to do so because of LD determinants shared with the responding cell. The sensitising cell pool thus seems to include essentially all human LD determinants.

Restimulation of a human pool-PLT cell with a xeno-

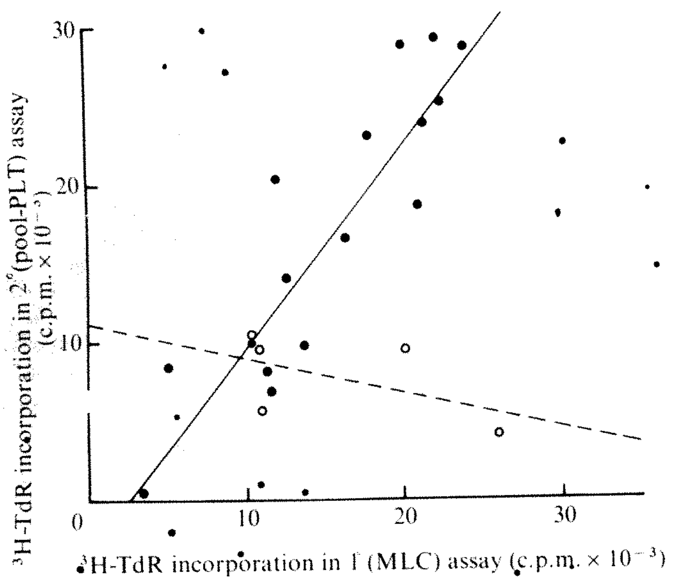

Fig. 1 Correlation of MLC and pool-PLT responses. Pool-PLT cells were generated and assayed as follows: 1×10^7 human responding cells were cultured with 1×10^7 irradiated, pooled human stimulating cells (consisting of 20 random donors) in upright flasks (Falcon No. 3013) in 30 ml RPMI 1640 medium supplemented with 25 mM HEPES buffer, penicillin, streptomycin and 10% heat-inactivated pooled human plasma. After 10 d the cells were collected, resuspended in fresh supplemented medium and distributed to flat-bottomed microtitre plates (Linbro No. IS-FB-96TC). 10^5 pool-PLT responding cells were restimulated with 2×10^5 irradiated human (●) or monkey (○) stimulating cells per well. Cultures were labelled with ^{3}H-TdR for 8 h on day 2 after restimulation. Primary MLCs were done using 10^5 fresh human responding cells and 2×10^5 irradiated human (●) or monkey (○) stimulating cells per well and were labelled for 8 h on day 5 of culture. ———, least squares regression line for human stimulating cells ($r = 0.89$, $P < 0.01$); ········, least squares regression line for monkey stimulating cells ($r = -0.55$, not significant).

geneic test cell should give a positive response if LD determinants on the xenogeneic test cell are shared with human LD determinants. Data for four species whose cells were tested for their ability to restimulate a pool-PLT cell (Table 1) show that in four experiments with five different pool-PLT cells, cells from all 10 rhesus monkeys tested caused significant restimulation. This restimulation was 44–135% of the average restimulation caused by a panel of random human test cells. There is a decreasing percentage of positive responses as well as percentage of restimulation by those cells that are positive. The primary MLC data show that those xenogeneic test cells that do not restimulate the pool-PLT cell still elicit a good primary MLC response from the same responding cell, indicating that the LD determinants recognised on those xenogeneic cells in a primary MLC are not represented in the human sensitising cell pool.

The data obtained with the pool-PLT test show that LD antigens are shared between species. The degree of sharing is quite extensive in rhesus monkeys, where all animals tested shared antigens with human cells, and less in dogs and cattle. In the 17 mouse strains we tested (11 independent and 6 recombinant H–2 haplotypes) there was no evidence for LD antigens shared with human cells.

Even in rhesus monkeys, where all animals so far tested have given positive secondary responses with pool-PLT cells, shared specificities do not seem to be the sole basis of recognition. In addition to the human cells shown in Fig. 1, five rhesus cells were also tested. All of these cells gave positive secondary responses, but the correlation between primary and secondary responses was poor ($r = -0.55$, not significantly different from zero), suggesting that there are additional LD determinants on the xenogeneic cells, not shared with human LD determinants, that are still recognised in a primary xenogeneic MLC.

Estimates of the frequency of initially responding cells in MLC to any one haplotype range from 1 to 6% (refs 6–8). As well as extensive polymorphism for LD antigens, in at least some cases the LD antigens controlled by two different haplotypes in the same species seem to be non-cross reactive[4,9]. We thus have to account for the ability of an individual animal to respond to all of the different LD antigens in its species, given the apparent clonal nature of this response[10,11].

That lymphocytes can also respond to xenogeneic cells in MLC[12,13], and that this response is specific and apparently directed towards the MHC LD antigens of the sensitising species[4], further compounds the problem in a quantitative sense. To overcome this difficulty it has been suggested that allogeneic recognition is unique[14] and, to the extent

Table 1 Pool PLT-cells restimulated by allogeneic or xenogeneic test cells*†

Xenogeneic species tested	48 h‡ pool-PLT	5 or 6 d‡ 1° MLC	No. of experiments (no. of pool-PLT cells)	No. positive§ / Total no. tested	Range of restimulation by positive cells‖ (median value)
Rhesus monkey	$AP_x + A_x$ 2,949¶	AA_x 1,005¶	4(5)	10/10	44–135% (71%)
	$+B_x$ 55,521	AB_x 37,692			
	$+X_x$ 30,418	AX_x 26,811			
	$+Y_x$ 24,183	AY_x 13,173			
Dog	$AP_x + A_x$ 1,943	AA_x 1,205	5(6)	13/14	11–97% (36%)
	$+B_x$ 26,194	AB_x 31,527			
	$+X_x$ 11,821	AY_x 17,367			
	$+Y_x$ 14,263	AY_x 12,973			
Cow	$AP_x + A_x$ 2,279	AA_x 1,173	5(5)	8/30	11–35% (17%)
	$+B_x$ 33,485	AB_x 31,723			
	$+X_x$ 12,279	AX_x 5,920			
	$+Y_x$ 4,509	AY_x 13,713			
Mouse	$AP_x + A_x$ 3,603	AA_x 3,299	5(7)	0/34	—
	$+B_x$ 24,835	AB_x 39,750			
	$+X_x$ 3,462	AX_x 19,450			
	$+Y_x$ 3,254	AY_x 14,470			

*All human responding cells are designated A, although different individuals were used as responding cells in each experiment.

†A_x, B_x: irradiated human stimulating cells.

X_x, Y_x: irradiated xenogeneic stimulating cells of species indicated.

‡Cultures were performed as in Fig. 1 legend, using 2×10^5 irradiated monkey or dog stimulating cells (peripheral blood lymphocytes), 8×10^5 irradiated cattle stimulating cells (peripheral blood lymphocytes), or 10×10^5 irradiated mouse stimulating cells (spleen cells depleted of erythrocytes by hypotonic shock) per well.

§All animals scored as positive gave restimulation values significantly different from autologous controls ($P < 0.05$).

‖Expressed as percentage of average restimulation by a random panel of human test restimulating cells.

¶c.p.m. ^{3}H-TdR incorporation.

that it occurs, xenogeneic recognition simply represents recognition of those xenogeneic antigens identical to, or cross-reactive with, the LD antigens of the responding species[15]. This would considerably limit the number of foreign MHC LD antigens that any animal would need to recognise. Our data indicate, however, that this is not the case. The very significant xenogeneic MLC seen between human and mouse cells, which seems to reflect specific recognitoin by human cells of mouse H-2 LD antigens, suggests that xenogeneic antigens can be recognised in MLC even when they are not cross-reactive with human LD antigens.

Thus we are left with the high frequency of initially responding cells in MLC, which must include specific recognition of allogeneic as well as non-cross-reactive xenogeneic LD antigens. Models that could explain this high frequency of initially responding cells have been discussed before[16-18]. One possibility is that the LD antigens coded for by different haplotypes within a species are to a large extent cross-reactive. The high frequency of responding cells in the rat system with the lack of any sharing of LD antigens in the few haplotypes tested could be the unusual case[9]. The very low number of MHC haplotypes existing in rat[19] is in marked contrast to mice[20] and humans[21], where cross-reactivity may be extensive. In fact, the high degree of LD sharing between humans and rhesus monkeys, and the still significant homologies with more distantly related species such as dog and cow, supports the concept that the large number of phenotypes that exist in all species may be based on the expression of different combinations of a limited number of determinants.

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Circulating immune complexes in normal human pregnancy

CIRCULATING antigen–antibody (AgAb) complexes are important pathogenic factors in various diseases. In addition to their inflammatory effect, immune complexes can interfere with cellular immunity. For instance, in cancer, the so-called blocking factors are apparently circulating AgAb complexes[1,2]. Factors interfering with cellular immunity specifically directed against placental and foetal antigens have also been described in pregnancy serum[3-5], and some data suggest a role of immune complexes as blocking factors in pregnancy[6]. We report here that circulating AgAb complexes are a constant feature of every normal pregnancy. This, of course, raises interesting questions as to the possible involvement of such complexes in the tolerance of the foetus by the mother.

Agglutination inhibition tests were carried out as before[7] using latex suspension provided by the Behring Institute (Marburg, FRG). However, human rheumatoid factor (RF) or Clq which failed to react with the immune complexes of pregnancy, were replaced by rabbit RF. RF was induced in rabbits by injecting 1 mg of autologous aggregated IgG mixed with complete Freund's adjuvant intradermally every 2 weeks. IgG was isolated by DEAE–cellulose chromatography and aggregated by heating at 63 °C for 10 min. For the isolation of RF, the animal sera were passed through a column of human IgG coupled to 6-aminohexyl-Sepharose 4B activated by glutaraldehyde[8]. The fraction eluted between 1 M and 2 M NH_4SCN was used after dialysis against saline. This fraction consisted almost exclusively of IgM.

Rabbit RF was inhibited by all sera from non-pregnant women ($N = 14$) at dilution titres ranging from $\frac{1}{4}$ to $\frac{1}{16}$, whereas the inhibition titres of 84 sera collected from 55 women at various stages of normal pregnancy before the 38th week, ranged from $\frac{1}{32}$ to 1/256 (Fig. 1). Those samples collected after the 38th week had a titre of $\frac{1}{16}$. The courses of two typical pregnancies showed an increase up to 1/128 of the RF inhibition titre with the advancement of pregnancy. At 3–4 weeks before delivery the inhibition titres dropped to levels observed in non-pregnant women.

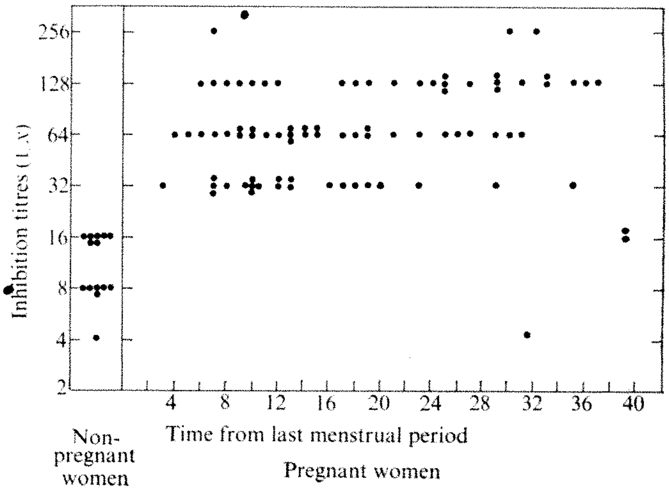
In an attempt to characterise the inhibiting factors, fresh sera from six pregnant women (gestation of 12–37 weeks) and with RF-inhibition titres ranging from $\frac{1}{64}$ to 1/256 were fractionated by gel filtration (Fig. 2).

In all sera from pregnant women, a high inhibitory activity towards RF was detected in the fractions corresponding to molecular sizes $\geqslant 10^6$. Sera from non-pregnant women were devoid of these large size inhibitory factors but, like sera from pregnant women, contained inhibitory factors eluted in the 7S region. In general, this inhibitory activity was maximal in the ascending slope of the peak of IgG (Fig. 2).

All sera from pregnant and non-pregnant women contained, in addition to 7S IgG, a small amount of this immunoglobulin eluted in a region corresponding to a molecular size ranging from 500,000 to several millions. On the average, 'heavy' IgG corresponding to 0.27% (s.d. 0.20) of our standard was recovered from 1 ml pregnancy serum, and 0.21% (s.d. 0.16) from 1 ml of control serum. These two quantities did not differ significantly. In one pregnant woman, both IgA and IgG were detected in the elution zone of IgM.

The C3 factor of complement was also found to be dis-

Fig. 1 Inhibitory activity toward rabbit RF of sera from pregnant and non-pregnant women.



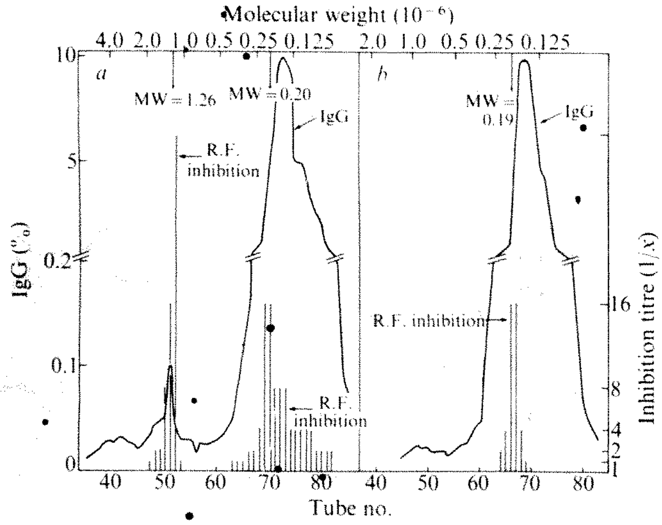


Fig. 2 Gel filtration of two serum samples (3 ml) on a calibrated Ultrogel AcA 22 column. *a*, Serum from a 24-week pregnant woman. *b*, Serum from a non-pregnant woman. The fractions of 5 ml were analysed by nephelometric immunossay of IgG (Technicon AIP system) and tested for their inhibitory activity towards rabbit RF. The concentrations of IgG were expressed in percentage of the IgG content of a pool of normal sera. Presence of IgA, IgM and C3 complement were followed similarly. Sera from age-matched non-pregnant women served as controls.

tributed in two fractions. The major one was detected, as was expected, in the ascending slope of the IgG peak, and the minor one in the elution zone of heavy IgG. In contrast to IgG, however, a highly significant difference ($P < 0.001$) was observed between the amounts of 'heavy' C3 occurring in pregnant and non-pregnant women. The pregnancy sera contained heavy C3 corresponding to 2.5% (s.d. 0.41) of our standard and the control sera 0.46% (s.d. 0.37).

Sometimes, the peak of heavy IgG or heavy C3 did not exactly match the peak of heavy inhibitory factors. Such a discrepancy was also observed in the fractionation of sera from patients with immune complex diseases. A possible cause could be the extreme sensitivity of RF for immune complexes of a certain size[7].

To determine whether the inhibitory factors of large molecular size corresponded to antigen–antibody complexes, the corresponding fractions were concentrated 10-fold by ultrafiltration, dialysed against 0.1 M HCl-glycine buffer, *p*H 3.0 to dissociate the complexes, and passed through a column of Sephadex G200 in the same buffer to separate the antigen from the antibody. Five peaks of material absorbing at 280 nm were obtained. After neutralisation by addition of 100 µl of 1 M Tris solution to the 5-ml fractions, they were analysed for IgG content by immunonephelometry and for their inhibitory activity toward RF. All fractions were found to be devoid of inhibitory factors. IgG was detected in the third fraction which was eluted in the position expected for a 7S protein.

A sample of 200 µl of each fraction was mixed with the same volume of each of the other fractions. After incubation at 37 °C for 30 min. and overnight at 4 °C, the 10 mixtures were titrated for their inhibitory activity. Only the mixture of the IgG-containing fraction with the second fraction gave an inhibition which was still detectable after a 1/512 dilution. This second fraction was eluted in a position suggesting a molecular weight of about 400,000. The resolution at acid *p*H of the inhibitory factors into five peaks was confirmed with a serum sample of another pregnant woman, and the identification of the material constituting each of these fractions is now in progress.

It is known that rabbit RF is capable of reacting with human IgG, especially when it is aggregated or involved in AgAb complexes[9]. Our gel filtration experiments clearly show that the increase of RF inhibition occurring during pregnancy is due to the appearance of factors of molecular sizes of between 500,000 and several millions. The presence of IgG and C3 in these heavy fractions and the dissociation-reassociation experiments leave little doubt about the AgAb complex nature of the heavy inhibitory factors. It remains to be explained, however, why rabbit RF was inhibited by heavy serum fractions from pregnant women and not from non-pregnant women, despite the fact that these fractions contained the same amount of IgG. This discrepancy might be due to a difference in the IgG subclass involved in the complexes. The hypothesis that rabbit RF would preferentially react with certain human subclasses of IgG is suggested by the elution pattern of the inhibitory factors detected in the 7S region. In sera from most pregnant and non-pregnant women, the maximal inhibition in the 7S region was found where material with a molecular size of about 170,000–200,000 was eluted. This inhibitory factor, therefore might correspond to IgG3, which has a molecular weight of 172,000 (ref. 10).

It has been reported that healthy individuals may possess IgG endowed with RF activity which can form an immune complex with other IgG molecules by a self-association mechanism[11]. The heavy IgG that we have detected in control sera presumably correspond to this type of complex. But, the type occurring during pregnancy seems clearly different since it contains larger amounts of C3 and an antigen which was not IgG but a substance with an estimated molecular size of about 400,000. We presume that this antigen originates from placental or foetal tissues.

The detection of circulating AgAb complexes in the serum of pregnant women opens several new perspectives. In gynaecology, the determination of immune complexes seems to be a possible test for monitoring the evolution of pregnancy. In pathology these immune complexes might be recognised as the pathogenic factors of preeclampsia, especially in view of the observation that deposits of IgG and C3 have been detected in the renal glomeruli of normal pregnant guinea pigs and mice[12]. In immunology, investigations of the blocking effect of immune complexes of pregnancy, and identification of the antigen and antibody involved may clarify the intriguing mechanism responsible for the immune tolerance of the mother toward the foetus.

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Y-antigen killing by T cells of women is restricted by HLA

CYTOTOXIC T cells are important in graft rejection and in the control of virus infections, but the mode of interaction of these cells with their targets, particularly in man remains unclear. It has been shown in mice that products of genes in the major histocompatibility complex (H-2) are involved in these interactions even when the cytotoxic T cells are specific for viral or non-H-2 antigens. This involvement is seen as the requirements that the target cell must express the specific non-H-2 antigen and in addition the same H-2, D or K region antigens as were present on the cells which initiated the immune response[1-4]. Cytotoxic T cells specific for viral or non-H-2 antigens are thus restricted in the targets that they can kill by the H-2 antigens on the targets. For example, cytotoxic T cells from H-2^b female mice suitably primed to the Y antigen of H-2^b males, will only kill cells from male mice carrying an H-2D region derived from H-2^b (refs 5, 6). This report is to our knowledge the first clear demonstration that a similar restriction occurs in man. We describe a situation in man where cytotoxic reactions specific for non-HLA antigens can only occur when target cells carry both the HLA-A2 antigen of the original sensitising cell and the non-HLA target determinant(s). The strong association with maleness suggests that one of the specific antigens involved was coded for by the Y chromosome.

Table 1 Killing pattern of cells from patient Reef. against her own target cells and those from other family members

Targets			HLA compatibility with patient	% Killing of CML	
Donor	♂	ac*	yes	63†	18‡
Patient	♀	ac	yes	−1	NT
Sib 5	♂	ac	yes	63	NT
Sib 3	♂	ad	no	53	13
Sib 4	♀	ad	no	31	NT
Mother	♀	cd	no	4	0

Table 1 shows percentage of chromium release of tests in which the patient's cells were tested against members of her family. NT, not tested.

*HLA haplotypes: a, A2, BW40, W6, CW3; b, A2, BW40, W6, CW3; c, A2, B12, W4, —; d, AW31, BW35, W6, CW4.

†Results after *in vitro* sensitisation to donor.

‡Results without previous *in vitro* sensitisation. MLRs between donor, patient and sib 5 were sufficiently low to exclude the possibility of a recombination between HLA-B and HLA-D.

Our study began with an observation that a female patient (Reef) who had been suffering from aplastic anaemia rejected a bone marrow graft from her HLA-identical brother. Before this rejection, a transient chimaerism of peripheral blood cells indicated that the graft had initially taken. Rejection was accompanied by a spontaneous recovery of the patient's bone marrow haemopoietic function, thus allowing further investigations. Peripheral blood lymphocytes from the patient were used as effector cells to measure the level of cytotoxicity against target cells from the patient's family and an unrelated panel. The patient's lymphocytes were first restimulated *in vitro* by incubating them for 6 d with irradiated stimulator cells from her HLA identical brother. Cytotoxicity was measured using an isotope release assay[7] with target cells which had been incubated with phytohaemagglutinin for 3 d and then labelled with sodium ^{51}chromate. Effector cells (70×10^4) and target cells (1×10^4) in RPM-1640 plus 20% heat-inactivated human AB serum were incubated for 4 h in round-bottomed microtitre plates. Plates were centrifuged for 5 min at 200g and the supernatant removed and counted in a γ-counter. Each cell combination was tested in triplicate. After subtraction of background the percentage of isotope release was calculated according to the following formula:

$$\frac{\text{experimental mean} - \text{mean of spontaneous release}}{\text{freeze–thaw mean (100\% release)} - \text{mean of spontaneous release}} \times 100 = \%\text{ kill}$$

In our initial studies performed 31 weeks after grafting, cytotoxicity was demonstrated against the donor's lymphocytes even when the patient's cells had not been restimulated *in vitro* (Table 1). This suggested that killing was to a non-HLA target as the donor was HLA identical to his sister. Three further experiments were performed using effector cells from the recipient sensitised to the donor and using cells from an unrelated panel as targets. HLA phenotypes are shown in Table 2.

Table 2 Positive and negative killing by cells from patient Reef against target cells from unrelated individuals

		HLA Phenotypes							%
		HLA-A		HLA-B		HLA-C		Sex	Kill
(Expt 1): week 42	1	$\underline{2}$*	W31	W35	$\underline{\text{W40}}$	$\underline{\text{CW3}}$	CW4	♂	30
	2	$\underline{2}$	11	W15	$\underline{\text{W40}}$	—	CW4	♂	33
	3	1	$\underline{2}$	8	$\underline{\text{W40}}$	—	NT	♂	28
	4	1	28	W17	W22	—	CW1	♂	−1
	5	9	11	5	W21	—	CW1	♂	−1
	6	$\underline{2}$	W31	$\underline{12}$	W35	—	CW4	♀	0
	7	1	$\underline{2}$	8	W22	—	—	♀	−1
	8	—	11	—	27	—	CW1	♀	−3
	9	11	W26	W16	W35	—	CW4	♀	−2
	10	1	W31	8	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♀	−1
Positive control (donor)		$\underline{2}$	$\underline{2}$	12	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♂	60
(Expt 2) week 52	11	1	2	8	W40	—	$\underline{\text{CW3}}$	♂	44
	12	$\underline{2}$	3	7	12	—	—	♂	47
	13	3	W30	W35	$\underline{\text{W40}}$		NT	♂	9
	14	$\underline{2}$	3	7	$\underline{\text{W40}}$		NT	♀	4
	15	$\underline{2}$	3	7	$\underline{\text{W40}}$		NT	♀	−1
	16	$\underline{2}$	3	7	$\underline{\text{W40}}$		NT	♀	3
	17	$\underline{2}$	9	5	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♀	15
	18	1	2	8	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♀	6
	19	1	$\underline{2}$	5	8	—	—	♀	−1
	20	$\underline{2}$	3	7	12	—	—	♀	3
	21	—	9	12	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♀	6
	22	11	W31	W15	W17	—	$\underline{\text{CW3}}$	♀	−1
Positive control (donor)		$\underline{2}$	$\underline{2}$	$\underline{12}$	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♂	48
(Expt 3) Week 72	23	—	3	18	27	CW2	CW4	♂	−3
	24	1	9	8	27	—	CW1	♂	0
	25	—	3	W17	18	—	—	♂	−3
	26	1	$\underline{2}$	5	5	—	CW4	♀	−2
	27	$\underline{2}$	W32	8	W15	—	$\underline{\text{CW3}}$	♀	−3
	28	3	W26	—	W35	—	CW4	♀	1
	29	10	28	W35	HR	—	CW4	♀	0
	30	—	9	7	W15	—	$\underline{\text{CW3}}$	♀	1
	31	1	3	W25	$\underline{\text{W40}}$	CW2	CW4	♀	−2
	32	1	9	18	W35	—	CW4	♀	−1
	33	1	3	—	W35	$\underline{\text{CW3}}$	CW4	♀	−1
	34	1	28	5	8	—	—	♀	−3
	35	3	W30	7	W17	—	$\underline{\text{CW3}}$	♀	−3
	36	1	9	8	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♀	0
Positive control (donor)		$\underline{2}$	$\underline{2}$	$\underline{12}$	$\underline{\text{W40}}$	—	$\underline{\text{CW3}}$	♂	13

NT, not tested.

*The HLA antigens compatible with patient Reef are underlined.

In several cases positive reactions were observed. The level of killing seemed to decline with time, and the gradual waning seemed to be associated with the patient's recovery. Family segregation studies were performed on the relatives of positive panel members. In three such studies the killing pattern failed to segregate with the HLA haplotype, again confirming that killing was directed to a non-HLA target or targets (Table 3).

We have searched for HLA restriction of killing, and a subsequent retrospective analysis revealed a strong association with HLA-A2. All targets that were killed carried the HLA-A2 antigen, although not all HLA-A2 cells were killed. The possibility that a split in HLA-A2 specificities was being recognised was excluded by the family studies (families 2 and 3, Table 3). Here the HLA-A2-bearing haplotype was associated with killing when expressed in one cell, but when expressed on another cell this haplotype was not associated with killing (family 2—c haplotype, family 3—a haplotype). Also, in family 3, no killing was associated with the antigen HLA-A28 which by serology was highly cross-reactive with HLA-A2.

Further analysis revealed that 15 out of 17 targets that were killed were male. Target cells from all fifteen of the HLA-A2 males were killed (Table 4) but cells from males not carrying HLA-A2 were not killed. Cells derived from two females were killed—both females were also HLA-A2 positive, but the level of killing was at a lower level than that observed with other positive reactions in the same test. This suggested that at least two specificities were involved, one associated with the Y chromosome exclusively and the other carried by a proportion of A2 females. One of the females that gave target cells showing positive killing was a sister of the patient (Table 1). The presence of the second system in the males will have been masked by the killing specific for the Y antigen. A clear example of the independent inheritance of an HLA-A2 gene product and the presumed Y product was shown in family 3 (Table 3). Here the HLA-A2 specificity inherited by the male child C1 was from the mother's a-haplotype. Cells from the two other male children were not killed.

Table 3 Pattern of killing of a non-HLA target by effector cells (raised between the patient Reef and her donor) tested on 3 families

Family 1 (week 64)		HLA haplotypes	Sex	% Kill
Target				
Mother	ab	1, 8, W6/2, W22, W6	♀	0
Father	cd	1, 8 W6/2, W40, W6	♂	23
C1	ac		♀	0
C2	ad		♂	19
C3	bc		♂	23
Family 2 (week 64)				
Mother	ab	3, 7, W6/1, 8, W6	♀	−1
Father	cd	2, 12, W4/2, W40, W6, CW4	♂	42
C1	ac		♂	34
C2	bc		♀	5
C3	ad		♂	41
C4	ac		♀	1
C5	bc		♀	3
Family 3 (week 72)				
Mother	ab	2, W40, W6, CW3/28, 12, W4	♀	1
Father	cd	1, 8, W6/2, W35, W6, CW4	♂	13
C1	ac		♂	15
C2	bc		♂	−1
C3	bc		♂	−2

In family 2 the female C2 inherited the A2 from the father and was not killed, but her brother who inherited the same A2 was killed. In family 3 the male C1 inherited the A2 from the mother and was killed, but C2 and C3 did not inherit A2 and were not killed. The weeks represent the time after bone marrow grafting that the family studies were performed. Compatibility with patient is underlined.

Table 4 Y chromosome linkage of non-HLA target and restriction of killing to HLA-A2 positive targets

		Sex of HLA-A2 targets	
		♂	♀
CML	+	15	2*
	−	0	17

*Level of killing was much lower than other positive targets in same experiment.

These results demonstrate that the cell-mediated cytotoxic reactions of the patient's lymphocytes are specific for non-HLA antigens but restricted by an HLA product in as much as only targets carrying HLA-A2 are lysed. This is very similar to the restriction seen with murine cytotoxic T cells reacting with non-H-2 antigens[1–6].

We have not yet unambiguously identified the effector mechanism in this human cell-mediated cytotoxicity system but feel it is not an antibody-dependent system as the cytotoxic reactions occurred in 20% heat-inactivated human AB serum. In these conditions it has been impossible to measure antibody dependent cell-mediated cytotoxicity (L. Doyer, personal communication). This is important as serum from the patient Reef, when tested against the panel members (Table 2) using the two-colour fluorescence technique[8], gave a positive reaction with some target cells that were positive in the cytotoxicity test.

Our observations of male-associated killing are analogous to those of Gordon *et al.* in the mouse[5,6]. In their case, cytotoxicity was only observed after *in vivo* sensitisation with a male skin allograft followed by *in vitro* priming. The clinical relevance of the Y-associated killing phenomenon is that it is probably an *in vitro* reflection of the known inefluence of sex on the incidence of graft against host disease in bone marrow transplantation between HLA identical siblings[9]. In kidney grafting, several workers have observed that female recipients of male grafts survived longer than female recipients of female grafts (ref. 10, and Kissmeyer-Nielsen, Opelz and Terasaki, personal communication). This was interpreted by Oliver to indicate that the Y-linked histocompatibility antigen may actively induce a prolongation of renal allograft survival in man. Although these results contrast with our own, they should be interpreted with caution as HLA-A2 positive combinations were not separately analysed, and some modes of immunisation to the Y-linked histocompatibility antigen may result in protective immunity.

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Selective cellular natural killing against human leukaemic T cells and thymus

NATURALLY-occurring antibodies against thymocytes, certain leukaemias and other tumour cells have been observed in various species including man[1,2]. In addition, natural cellular immunity—also known as cellular natural killing (CNK)—has been described recently in man[3–5] and in mice[6–9]. The possible role of CNK in tumour surveillance and the nature of the effector cells are presently subject to extensive research. Relatively little is known, however, about the nature and sensitivity of the target cells in relation to CNK. We report here that human leukaemic T cells and human thymocytes are highly sensitive to CNK by normal human peripheral blood and that B cell lines are relatively insensitive. The response seems to be unrelated to HLA and expressed variably by different individuals.

Table 1 Characteristics of tissue cultured human lymphoid cell lines

Cell line	HLA phenotype	Cell type	Cell origin	References
SB	1,2,12,27	B	Leukaemic	10
HSB	1,2,12,27	T	Leukaemic	10
CCRF–CEM	1,w33,6,8	T	Leukaemic	11
JY*	2,7	B	Normal	—
PY*	2,7	B	Normal	—
HL*	2,w35	B	Normal	—
TLCL 23-12-1†	w31,w24,5,w40	B	Normal	—

*Cell lines originally established in culture and kindly provided by Dr D. Mann.

†Cell line originally established in culture and kindly provided by Dr R. Gatti.

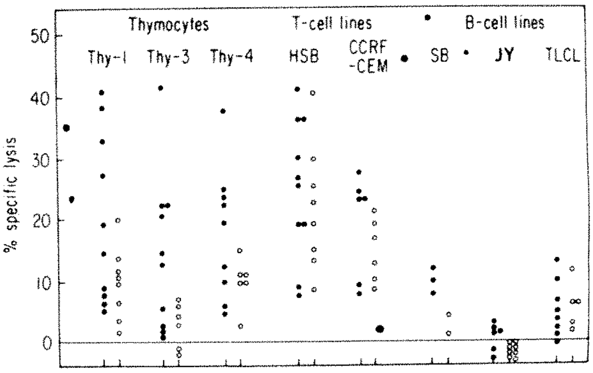

Fig. 1 Distribution of CNK of 18 individuals tested against thymocytes, T- and B-cell lines. A total of 18 samples of human mononuclear cells were tested in several experiments against ^{51}Cr-labelled PHA-stimulated thymocytes of three individuals (Thy-1, Thy-3 and Thy-4), against two T-cell lines (HSB and CCRF-CEM) and against three B-cell lines (SB, JY and TLCL). The ratio of effector cells to targets was 50:1 in all experiments. Activity is expressed as % specific lysis in a 4-h assay, performed as for Table 2. ●, Male donors ; ○, female donors.

Tumour cells with T- and B-cell characteristics as described in Table 1 were cultured in minimal essential medium (MEM) supplemented with 10% foetal calf serum (FCS). HSB and SB were obtained from the same individual; HSB is a leukaemic T-cell line, SB is a B cell established from the patient's peripheral blood leukocytes (PBL) in culture. CCRF-CEM is another leukaemic T cell and JY, TLCL, PY and HL are established B-cell lines. Thymocytes were obtained from patients undergoing open-heart surgery. The cell lines and phytohaemagglutinin (PHA) stimulated thymocytes were labelled with ^{51}Cr and used as target cells in a 4-h modified microcytotoxicity assay[7]. Mononuclear cells from normal individuals were separated from heparinised whole blood on Ficoll–Hypaque as described by Boyum[12] and served as effector cells. To test whether human T- and B-cell lines differ in their susceptibility to lysis, they were labelled with ^{51}Cr and incubated with normal PBL. Table 2 summarises the results of one such experiment showing that three donors of differing HLA phenotype exhibited high cytotoxic activity against the leukaemic HSB and CCRF-CEM T-cell lines in all ratios tested and had comparatively low activity against SB and JY B-cell lines. This 'cross-reactivity' is discussed in more detail below. Although HSB and SB were obtained from the same individual and are therefore identical for their HLA and presumably for other cell-surface HLA loci (see Table 1), their reactivities in CNK differ markedly. The other B-cell lines studied—TLCL, PY and HL—behaved similarly to SB and JY (data concerning PY and HL are not presented), while CCRF-CEM, the other leukaemic T-cell line tested, was as sensitive to CNK as HSB. CCRF-CEM and HSB are from different and unrelated subjects. Since leukaemic T cells are thought to be thymus derived it was of interest to investigate the sensitivity of human thymocytes as targets in our cytotoxic assay. Fresh thymocytes used as targets on the same day they were obtained from surgery or cryopreserved thawed thymocytes were unsuitable targets owing to poor ^{51}Cr incorporation and rapid spontaneous release. Prestimulation with PHA (PHA HA 16/17, Wellcome) at 1 μg ml^{-1} for 3 d or less resulted, however, in a highly viable population of cells incorporating up to 20-fold more ^{51}Cr than non-PHA-stimulated cultures on day 0. These cells were then used as targets in the cytotoxic assays. Blood samples from 18 normal individuals were tested against thymocytes obtained from 4 HLA different individuals and prestimulated with PHA for 3 d (Thy-1, Thy-2, Thy-3, Thy-4). All donors tested exhibited significant cytotoxic activity against all thymocyte targets. Table 3 shows the results of four representative donors only. The selectivity of the CNK was demonstrated by the lack of activity against JY, a B cell line. It should be noted that all the B cell lines used in this study served as excellent targets when tested with *in vitro* allogenically sensitised human PBL (A.O., unpublished data). To our knowledge this is a unique and first example demonstrating CNK against 'normal' cells in general and thymocytes

Table 2 Selective CNK activity against T- and B-cell lines

		% Specific lysis*											
		HSB			CCRF–CEM			SB			JY		
Effector cells	HLA	25 : 1	12 : 1	6 : 1	25 : 1	12 : 1	6 : 1	25 : 1	12 : 1	6 : 1	25 : 1	12 : 1	6 : 1
AO	3,w24,12	29.0	24.3	15.8	21.1	15.5	11.6	7.9	6.7	5.5	6.4	4.9	−1.8
HK	28,w24,14,18	31.7	24.0	17.1	22.6	18.9	11.4	8.8	4.8	7.3	5.3	3.3	1.4
BS	3,7,11,14	22.4	16.0	13.5	18.0	15.6	14.8	6.7	3.9	2.1	−2.9	ND	ND
Spontaneous release			(14.3)			(32.7)			(29.7)			(30.9)	

*Target cells were labelled with 100 μCi of ^{51}Cr for 2 h at 37 °C, washed three times and resuspended in MEM 10% FCS to 1×10^5 per ml. The cytotoxic assay was performed in Cooke round-bottom microtitre plates by mixing 100 μl of effector cells with 100 μl of target cells and incubated for 4 h at 37 °C in a humidified CO_2 incubator. The reaction was terminated by centrifugation at 250*g* for 10 min and removing 100 μl of supernates from all wells to be counted in a well-type γ-counter. Specific cytolysis was calculated as $[(E-SR)/\text{Max})] \times 100$ where E is c.p.m. of experimental wells; SR is spontaneous release from targets incubated in medium alone and Max is maximal release from targets after hypotonic lysis. The standard deviation of triplicate cultures was ±2% of the mean. ND, not determined.

Table 3 Lysis of PHA-stimulated thymocytes by normal PBL

Effector cells	HLA	1.10w37 Thy-1	2,12,29,w15 Thy-2	2,28,w40,12 Thy-3	10,w30,29,13 Thy-4	1,2,12,27 HSB	2,7 JY	2,12,29,T5 AMT1,	3,w24,12 AO
		% Specific lysis							
KT	2,5	32.8	27.0	41.7	19.3	26.0	−2.2	ND	ND
AO	3,w24,12	27.5	18.0	20.2	22.6	29.8	−0.1	0.3	−4.7
DBA	2,5,1.7	40.8	12.2	22.3	ND	36.0	0.9	ND	ND
KY	5,9,w26,w40	38.5	ND	22.5	37.8	41.0	1.0	1.4	−3.0
Spontaneous release		(18.8)	(24.8)	(20.0)	(22.7)	(14.3)	(21.2)	(24.5)	(27.8)

Thymocytes from four individuals and PBL of two donors (A.M. and A.O.) were stimulated in culture with PHA at 1 μg ml^{-1} for 3 d, then labelled with ^{51}Cr and used as target cells in the cytotoxic assay. Assay as for Table 2. The ratio of effector to target cell was 50: 1 throughout.

in particular. Normal human PBL from two individuals (A.M. and A.O.) stimulated by PHA *in vitro* were not lysed by lymphocytes of normal individuals, confirming an earlier observation[3]. By screening normal individuals for their activity in CNK it became obvious that different values of specific lysis are obtained with different donors. Figure 1 illustrates this variability when normal donors were tested against three thymocyte populations, two T-cell lines and three B-cell lines. Using thymocytes as targets, cytotoxic activity was 0–42%, compared with 10–40% with the T-cell lines and < 0–15% with the B-cell lines. It seems, therefore, that lymphocytes from different individuals have different killing activities. The range of lytic capacity extends from low to high and does not seem to fall into distinct categories. The difference in the killing activity between males and females seems to be distinct only when thymus target cells are used with males being more reactive. We have noticed that individuals with high reactivity against thymus and T-cell lines occasionally show 'cross-reactivity' against B-cell lines as shown above (see Table 2). Low killers have a more restricted 'specificity' — killing thymus and T cell lines only.

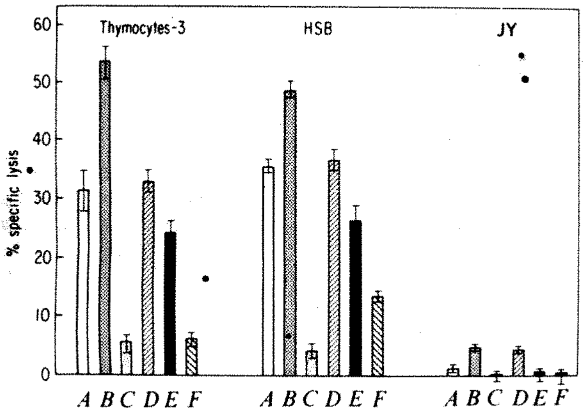

Fig. 2 Partial characterisation of the effector cell in CNK. Normal human mononuclear cells were subjected to various depletion and separation techniques (see below) resulting in populations of cells with different degrees of CNK when tested against ^{51}Cr-labelled PHA-stimulated thymocytes, HSB and JY cell lines. The ratio of effector cells to targets was 25:1. Activity is expressed as % specific lysis in a 4-h assay (as for Table 2). Spontaneous release of the target cells used in this experiment was as follows: Thy-3, 9.3%; HSB, 21.3%; JY 23.8%. *A*, Normal unfractioned cells; *B*, PBL depleted of phagocytic cells following ingestion of carbonyl iron (1 g per 5×10^6 cells incubated at 37 °C for 20 min); T-cell enriched fraction was obtained by incubating equal volumes of neuraminidase (Behring Diagnostics) treated sheep red cells (SRC) and lymphocytes for 10 min at 37 °C followed by a centrifugation at 200*g* for 5 min and incubation in ice for 1 h. The pellet was then gently resuspended in medium and rosetted cells were separated from non-rosetted cells by the use of a Ficoll–Hypaque gradient. *C*, SRC of the rosetted fraction were lysed with an anti-SRC haemolysin at a 1:10 dilution and fresh undiluted human serum; *D*, non-rosetted cells with neuraminidase treated SRC after Ficoll–Hypaque gradient separation; *E*, F_c positive cells were separated from F_c negative cells based on their ability to bind to SRC anti-SRC complexes bound to poly-L-lysine pretreated plastic dishes following the technique of Hsia *et al.*[13]; *F*, F_c negative cells were obtained by collecting cells not adhering to the antigen–antibody complexes described above.

The nature of the effector cell in CNK is a controversial issue. We have partially characterised them in our system, in limited and preliminary experiments. Different depletion and fractionation procedures resulted in populations of cells with different degrees of CNK activity. Similar profiles were obtained by testing these populations against either thymocytes or HSB target cells. The experiment illustrated in Fig. 2 shows the following. (1) Removal of phagocytic cells by carbonyl iron and magnet enriched the cytotoxic activity against both thymus and HSB targets. (2) Using sheep red blood cells as indicators, the rosetted cells, presumably T cells, were almost inactive. (3) The non-rosetted cells retained their activity. (4) Cells possessing a receptor for the F_c portion of immunoglobulin (F_c+) retained most of the activity against both thymus and HSB cells. And (5), F_c− cells had diminished activity but were by no means depleted of effector function. It therefore seems that the effector cell is not a mature T cell, nor a macrophage but is F_c+.

We have, therefore, demonstrated cellular immunity of normal individuals against T-cell lines and PHA stimulated thymocytes and a relative lack of activity against B-cell lines even when the T- and B-cell lines came from the same individual. The CNK activity that we observe also does not seem to correlate with HLA. This is in contrast to reports[14,15] of H-2 specificity in a mouse system. Our results suggesting that males have higher CNK activity than females against thymus cells, are in agreement with the findings of Santoli *et al.*[16] with human (non-lymphoid) cell lines as targets. It is tempting to speculate that our *in vitro* finding of selective CNK against leukaemic T cells but not against B-cell lines may be reflected *in vivo* by the relatively low incidence of acute lymphocytic leukaemias usually associated with cells of the T-axis characteristics[17]. Moreover, since T cells are known to be poor antigenic stimulators in MLC[18], it would be logical to propose a different naturally occurring immune mechanism controlling proliferation of immature thymocytes and leukaemic T cells. We suggest that CNK might function in such a surveillance pathway and may also discriminate between mature peripheral T cells on the one hand and immature or leukaemic T cells on the other.

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Carcinogen-induced mutations at two separate genetic loci are not enhanced by leukaemia virus infection

THE interrelationship between cellular mutagenesis and carcinogenesis has been studied primarily by measuring these two events in separate target systems using chemicals with known biological activity[1]. Most carcinogens, in appropriate experimental conditions, induce mutations in prokaryotic[2] and eukaryotic[3] test systems. There are, however, some well known exceptions. Some nucleic acid base analogues and the acridines, which are excellent mutagens, are not known to be carcinogenic *in vivo*. Similarly, asbestoid minerals and some metallic salts which are potent carcinogens do not produce gene mutation. We describe here a study of the relationship between transformation and mutation with different experimental approaches, and show that carcinogen-induced mutations at two separate genetic loci are not enhanced by leukaemia virus infection.

The use of Fischer rat embryo (FRE) cells for *in vitro* assay of chemical carcinogens has been well documented[4]. In appropriate conditions, most of the ultimate carcinogens and procarcinogens transform FRE cells synergistically only after being preinfected with murine leukaemia virus (MuLV)[7]. Since infection by MuLV so profoundly affects the expression of chemically induced transformation in this sytem, it was of interest to determine the effect of MuLV on the carcinogen-induced cellular mutagenesis in FRE cells.

A clonal subpopulation of the FRE cell line F1706 used in these studies (hereafter designated as M81 cells) has been described previously[6]. Logarithmically-growing M81 cells were infected with 10^4 XC plaque-forming units of the Rauscher strain of MuLV (RLV). RLV-infected or uninfected M81 cells were treated with various doses of the potent carcinogen–mutagen, 4-nitroquinoline-1-oxide (NQO), for 1 h, washed and incubated at 37 °C in the growth medium for 72 h (expression time). The post-expression cells from each treatment group (M81+acetone; M81+RLV+acetone; M81+NQO; M81+RLV+NQO) were then used for the determination of DNA polymerase activity[7], NQO-induced transformation[8,9], and mutagenesis at two separate genetic loci.

To determine transformation in M81 cells, a previously described 'horizontal–vertical' assay[4] which involves both morphologic criterion and the loss of anchorage dependence (soft agar assay) was used. Colonies in semi-solid agar and morphologically transformed foci in liquid medium first appeared in the culture flasks after 5 and 7 subcultures respectively in the dually treated group (RLV and NQO 0.1 μg ml^{-1}). After several more subcultures, the focal characteristic of morphological transformation was lost and the entire culture appearedto be transformed. No morphological transformation or loss of anchorage dependence could be detected in the other treatment groups (M81+acetone; M81+RLV+acetone; M81+NQO 0.1 μg ml^{-1}) before the experiment was terminated at the tenth subculture. The transformation of RLV-infected FRE cells by NQO confirms the results previously reported by other investigators[10,11]. It should be noted, however, that due to the number of subcultures involved before there was evidence of transformation, enumeration of the foci or the soft agar colony counts should not be used quantitatively.

The number of mutants in M81 cells was determined by plating the post-expression cells at low densities and then, after 72 h, adding appropriate selecting media. For mutation in the recessive locus governing the expression of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT: EC 2·4·2·8), 8-azaguanine (AZ) was used for selection and all experiments were performed with a single lot of commercial foetal bovine serum. The frequency of the appearance of AZ-resistant colonies per plate followed a typical Poisson distribution ($\lambda = 0.1$) and were verified by χ^2 test ($P > 0.99$). Table 1 shows the number of AZ-resistant phenotypes per 10^5 survivors in a typical experiment 72 h after chemical treatment (NQO 0.1 μ ml^{-1}, 1 h). A statistically significant increase ($P < 0.01$) over the spontaneous mutation rate was clearly demonstrated with NQO treatment in both RLV-infected and uninfected groups. Using a standard one-tailed test of significance for the observed differences between two Poisson variables[12], however, there was no significant difference ($P < 0.05$) between the number of spontaneous AZ-resistant colonies in the RLV-infected or uninfected M81 cells. Similarly, RLV infection did

Table 1 Transformation and 8-azaguanine resistant phenotypes in RLV$^+$ and RLV$^-$ post-expression FRE cells

	RLV$^-$		RLV$^+$	
Carcinogen	No addition	0.1 μg NQO	No addition	0.1 μg NQO
DNA polymerase activity	0	0.5	48.6	55.05
Transformation (subculture no.)	− (P_{10})	− (P_{10})	− (P_{10})	+ (P_5)
No. of replicate plates for AZ-resistance assay	16	20	20	20
% Relative PE (at 72 h)	100	52	188	125
Total no. of AZ-resistant colonies	3	27	8	65
% Plates containing following groups of resistant colonies:				
0	81	40	65	5
1	19	20	30	0
2 or 3	0	30	5	60
4 or more	0	10	0	35
Mean no. of resistant colonies per 10^5 survivors	1.77	19.20	1.39	19

Virion-associated DNA polymerase activity in the cell culture fluids were measured using an established procedure[7] with synthetic template primer dT_{12} rA and expressed as pmol of acid-precipitable H^3-TMP per h per ml of supernatant collected at 72 h. The input radioactivity used for polymerase assay contained 1930 c.p.m. per picomole of TTP. Transformation assays[8,9] were performed for 10 subcultures after NQO treatment when the experiment was terminated; results were expressed as either positive or negative at indicated numbers of subculture. For AZ-resistance assay, 1×10^5 M81 cells were plated in 90-mm Falcon Petri dishes 72 h after NQO treatment in EMEM growth medium (Earle's minimal essential medium with 10% newborn calf serum and antibiotics). After 3 d, growth media supplemented with AZ 30 μg ml^{-1} (selective media) was added. The cultures were subsequently fed every third day with selective media and the dishes were stained with Giemsa on the fifteenth day for enumeration of AZ-resistant colonies. Plating efficiency of each treatment group was determined simultaneously by plating 5×10^2 post-expression cells in growth media without AZ. These parameters for selection of AZ-resistant cells were optimal for M81 cells in pilot experimentations performed for standardisation of the assay procedure.

Table 2 Reconstruction experiment with HGPRT⁻ subclones

Input cell mixing ratio (AZ-mutants/M81 RLV⁺)	Carcinogen treatment	% Absolute PE (72 h)	Mean no. of recovered AZ-resistant colonies per 10^5 survivors
$0/10^5$	None	46.2	1.27
	NQO 0.1 μg ml⁻¹	43.4	18.49
10 AZ–1/10^5	None	52	25
	NQO 0.1 μg ml⁻¹	49	44.90
10 AZ–2/10^5	None	56.6	24.91
	NQO 0.1 μg ml⁻¹	52.8	45.83

5×10^2 AZ-resistant mutants, AZ-1 or AZ-2 were mixed with 5×10^6 RLV infected M81 cells. 2×10^6 mixed cells were plated in T-flasks and incubated at 37 °C. After 6 h, cells were washed and treated in NQO 0.1 μg ml⁻¹ or acetone for 1 h. Each flask was then washed and incubated at 37 °C for 72 h. Selection of the AZ-resistant cells in the mixed population was performed as for Table 1 in order to determine the efficiency of the selection technique.

not significantly alter the outcome of NQO-induced mutation. Thus, although treatment with NQO produced an increase of AZ-resistant cells in both infected or uninfected groups, infection of the target cells with RLV did not alter the spontaneous or induced rate of mutation for the appearance of the drug-resistant phenotype. Several of the AZ-resistant colonies were isolated for further characterisation. Two of these clones, AZ-1 and AZ-2, were propagated for at least 50 generations in nonselective growth medium and still retained their high resistance to AZ (PE 18 and 19.6 respectively and sensitivity (PE < 0.01%) to THMG supplemented growth medium[13]. A reconstruction experiment using these two resistant subpopulations was performed to check the validity of the calculations used for the determination of the HGPRT⁻phenotypes. The results of the reconstruction experiment (Table 2) indicate that the estimates of the proportions of AZ-resistant cells, based on the selection procedure, are within the same order of magnitude of the actual proportions used for mixing resistant cells with the the parent M81 population.

Since the phenotypic expression of AZ-resistance can vary several-fold due to minor variations in the experimental protocols, it was considered necessary to determine mutation at another locus. Resistance to the synthetic steroid, ouabain, involves mutation of the gene controlling the expression of cell membrane-bound enzyme $Na^+/K^+\text{–}Mg^{2+}$-dependent adenosine triphosphatase (ATPase: EC 3.6.1.3). The mutation is codominant and a single step selection can be performed over a wide range of input cell concentrations[14]. To evaluate the effect of RLV infection on the NQO-induced mutation at the ATPase locus, subconfluent cultures were treated with different doses of NQO and the number of ouabain-resistant phenotypes was determined after 72 h expression period.

Figure 1 shows the dose–response pattern for the appearance of ouabain resistant colonies. It can be seen that over a wide range of NQO input doses, a linear response for the appearance of the ouabain resistant cells is evident in both RLV-infected or uninfected M81 cells. For uninfected cells, the regression line can be best described by the equation $F^- = 19.51 \times \mu g\ ml^{-1}\ NQO + 0.27$, while for RLV⁺ cells the linear relationship is $F^+ = 18.2 \times \mu g\ ml^{-1}\ NQO + 0.81$, where F^- and F^+ represent mutation frequencies per 10^5 survivors. However, the slopes of the two regression lines (19.51 and 18.2, respectively) are not significantly different by Student's *t* test ($P \gg 0.05$). Thus, RLV infection again failed to enhance the mutagenic response of M81 cells at any of the NQO doses tested. As before, several ouabain-resistant colonies were isolated, cultured in nonselective medium and these subpopulations were tested in a regular or K⁺-deficient

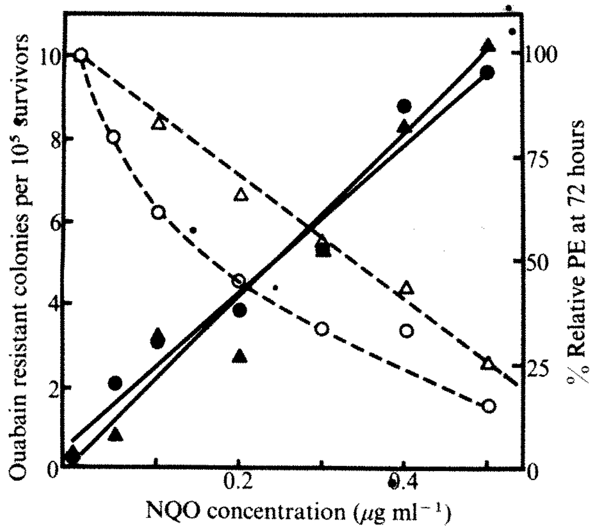

Fig. 1 Percentage survival and ouabain-resistant colonies per survivor in FRE cells as a function of input NQO dose. △, M81 relative PE (%); ○, M81+RLV relative PE (%). ▲, Ouabain-resistant colonies in 10^5 surviving M81 cells; ●, ouabain-resistant colonies in 10^5 surviving M81+RLV cells. For ouabain resistance tests, 5×10^5 cells were plated in 90-mm Falcon Petri dishes 72 h after NQO treatment in EMEM growth medium. Ouabain (2.5 mM) was added to the medium after 3 d and dishes were incubated at 37 °C without any subsequent feeding and stained with Giemsa on the fifteenth or eighteenth day for counting the drug-resistant colonies. Each point represents a mean obtained from 10 dishes, except that 20 replicates were used for groups without NQO treatment. It should be noted that normalised relative survival of each test group was based on plating efficiencies obtained at 72 h without NQO treatment (five replicates) and all PEs were performed simultaneously with the ouabain selection procedure. Solid symbols (ouabain resistant colonies) represent means of the experimentally obtained data and solid lines connecting them represent calculated regression lines. See text for regression equations for each line.

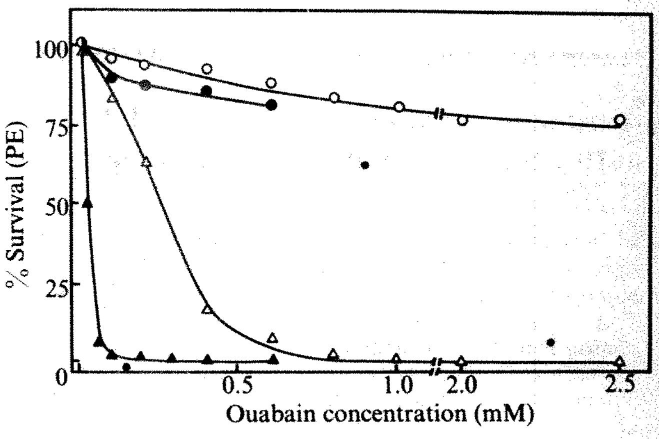

Fig. 2 Relative plating efficiencies of M81+RLV cells and an ouabain-resistant subclone (M418) as a function of ouabain concentration. △, M81+RLV cells cultivated in EMEM (55%); ▲, M81+RLV cells in K⁺-deficient EMEM (27%); ○, M418 cells in EMEM (58%); ●, M418 cells in K⁺-deficient EMEM (49%). The PE of each experimental cell line in ouabain was determined as described in the legend to Fig. 1, except that 10% dialysed calf serum (ds) was used in conjunction with ouabain treatment. Cells were plated at three different concentrations (5×10^2; 5×10^3; and 1×10^4 per plate) in triplicate and accordingly zero PE simply reflects that no surviving colonies were observed at the highest plated cell concentration. The average % PE for each of the four experimental groups without ouabain, which has been used as a basis for the normalisation of the data (100%) are given in parentheses.

selective medium for ouabain resistance. The toxicity of ouabain for M81 clones, as manifested by the reduction in the number of surviving colonies, is dependent on K$^+$ content of the medium. A typical toxicity pattern for one of the resistant subpopulations (M418) is shown in Fig. 2.

Our results indicate that although RLV infection profoundly affects the expression of transformation in FRE cells, mutation rates at two unlinked genetic loci—one co-dominant and the other recessive—are not affected by the virus. Perhaps a specific, privileged role of the ecotropic MuLV on the promotion of cellular transformation at the genetic level can be envisioned which is not applicable to other non-specific and random genetic events like mutation induced by carcinogens. In this context, it is interesting to note that chemical promoters of transformation, such as phorbol esters[15], which presumably act epigenetically, has recently been shown to also enhance the cellular mutagenesis rates[16].

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Antibodies to major histocompatibility antigens produced by hybrid cell lines

FUSION between myeloma cells and spleen cells from immunised donors has been shown to be a successful method of deriving homogeneous anti-SRBC (anti-sheep red blood cell) and anti-TNP antibodies[1,2]. One of the most powerful features of this approach is that, by cloning, one may easily derive cell lines synthesising monoclonal antibodies despite using non-purified immunogens. The multiple components of a heterogeneous population of hybrid cells are resolved by cloning techniques. This feature makes the system a very powerful tool in the study of complex antigenic structures. The established cell lines offer the further advantage of unlimited permanent supply of material, and the possibility of worldwide standardisation.

We report here the derivation of lines of hybrid cells secreting antibody to rat major histocompatibility antigens. The hybrids were made by fusion of a mouse myeloma cell line[1], P3-X63-Ag8, with spleen cells of AO strain rats immunised against DA strain antigens. In previous experiments we used Sendai virus as a fusing agent. In the experiments described here we used polyethylene glycol (PEG). Fusion of animal cells with PEG is becoming a method of choice. Various protocols have been described in detail, usually for cells growing as monolayers[3–5]. We adopted some of these suggestions, which take special care with the rate of addition of reagents, time and temperature of incubations, molecular weight of PEG and so on (see legend to Fig. 1 for details). After the hybridisation the preparation was divided in Linbro plates, each 2-ml well containing about 2×10^6 spleen cells. Vigorous growth was observed in all the wells indicating the presence of multiple hybrid clones in each well. The supernatants of each well were tested for their ability to mediate the complement-dependent lysis of ^{51}Cr-labelled red cells and lymphocytes from DA strain donors. Of 113 cultures from two independent fusions 107 contained significant lytic activity. The most active cultures were further grown to larger volumes. During growth, some cultures gradually lost activity, whereas others retained or even increased their lytic activity. This presumably reflects the clonal complexity of the cultures—after a certain period, dominant clones (fastest growing) are likely to take over regardless of their antibody activity.

The individual cultures displayed lytic activities which, in some cases, could be shown to be distinctive. For instance, hybrid R2/10 lysed 100% of DA red cells, while R3/13 never lysed more than about 55%, although the titres of the two supernatants were very similar on this target (Fig. 1). Furthermore, the R3/13 supernatants tested for lympholytic activity showed a markedly higher titre than R2/10.

After about 2 months of growth, hybrid R3/12 was cloned in soft agar and 14 individual clones were selected for further studies. Supernatants from six of these were positive in three tests for specific immunological activity, while the eight others were negative in all three tests (Fig. 1). Clones R3/13-6 and R3/13-8 had lower antibody titres than the other positive clones. The Ig produced by the isolated clones was analysed by isoelectric focusing and SDS–polyacrylamide gel electrophoresis of ^{14}C-lysine-labelled secreted products (Fig. 2). In all cases the characteristic pattern of products of the parental X63 cells due to MOPC 21 IgG1 (κ) was present. All positive clones had very similar patterns of additional bands which clearly established the presence of extra components, presumably responsible for the antibody activity. These extra components were seen in IEF as a few extra bands (Fig. 2) and a background of radioactivity. A similar background of radioactivity has been observed with another plasma cell hybrid (Sp2/HLGK, ref. 2). The presence of the background in this latter case was associated with the presence of a specific IgG2b, an euglobulin which precipitates at low ionic strength (D. Secher, unpublished). Electrophoresis in reducing SDS–polyacrylamide gels shows the presence of an additional light chain in all cases. The putative specific heavy chain co-migrates with the myeloma $\gamma 1$ chain but can be separated on much longer runs. A clear indication of clonal heterogeneity was observed in clone R3/13-6 with a weak band in the position of a μ chain and an unusually high molecular weight putative L-chain (Fig. 2). This clonal heterogeneity may explain the low titre of its supernatant (Fig. 1). The reason for the low titre of clone 8 has not been investigated further.

Specifically positive clone supernatants from R3/13 were tested in various ways to demonstrate that the antigenic target for the antibody was associated with the rat major histocompatibility complex, Ag-B. Supernatants were tested against target cells of four inbred rat strains each carrying a different Ag-B haplotype including AO, the strain of the parental spleen cells used for fusion (Table 1). By ^{51}Cr release from target lymphocytes and by binding to erythrocytes, clone supernatants were reactive only against cells from the original immunising strain, DA. Chromium release data for clone supernatants were compared with two dilutions of a standard AO anti-DA immune serum. This antiserum, which maintains cytotoxic activity at a plateau up to a dilution of about 1/6,000 for DA lymphocytes, cross-reacts detectably on HO targets at about 1/800 and on AGUS targets at about 1/100. Since R3/13 clone product has a plateau cytotoxic titre of 1/384 for DA cells (Fig. 3), detectable cross-reactions would be expected at 1/48 on HO and at 1/6 on AGUS if the specificity of the clone product were similar to that of the AO anti-DA antiserum. The absence of any detectable cross reactions at 1/6 (maximum concentration tested) shows that the R3/13 clone product is much more specific than the immune serum.

Since positive clone supernatants were not reactive against HO

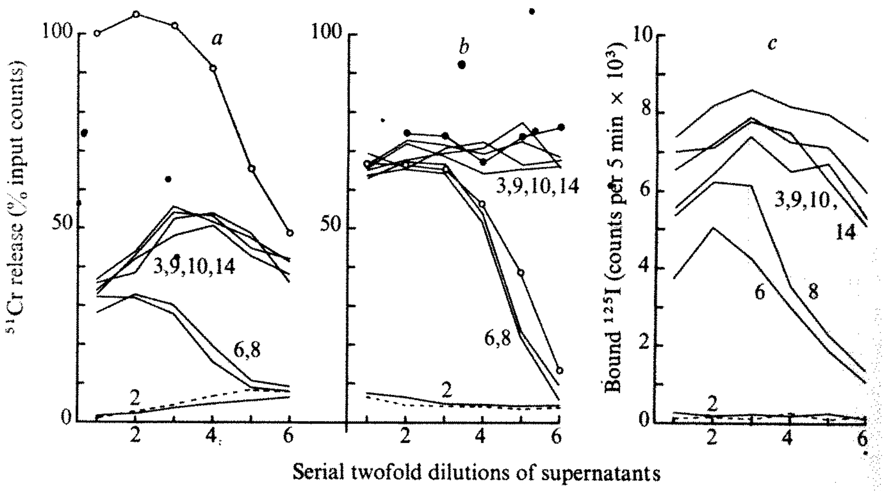

Fig. 1 Immunological activity of spent medium from plasma cell hybrids. Hybrid culture R3/13 was cloned in soft agar and 14 individual clones (R3/13-1 to R3/13-14) removed from the agar and grown separately. Supernatants from numbered R3/13 clones (—) were tested in three assays for immunological activity. Supernatant from the uncloned hybrid R2/10 (○) was included for comparison in two assays. Supernatants from clones R3/13-3, 6, 8, 9, 10, 14 were positive in all three assays, and from clones R3/13-1, 2, 4, 5, 7, 11, 12, 13 were negative in all three assays. Of the negative clones, results are shown only for R3/13-2. DMM served as negative control (– – –). *a*, Complement-dependent lysis of DA erythrocytes assayed by ^{51}Cr release. 10^8 fresh washed erythrocytes were labelled for 90 min at 37° with ^{51}Cr-sodium chromate (CJS • 4, Radiochemical Centre, Amersham) at approximately 5 mCi ml^{-1} in 0.025 ml. Assays were performed in V-bottomed microtitre trays in a final volume of 0.075 ml consisting of equal volumes of diluted clone supernatant or medium, washed labelled erythrocytes at a concentration of 5×10^7 ml^{-1} in Dulbecco's phosphate buffered saline (PBS), and guinea pig serum (absorbed with rat erythrocytes) diluted 1:1 in Dulbecco's PBS. Trays were incubated for 60 min at 37 °C. After adding 0.1 ml PBS to each well, trays were spun 10 min at 400*g* and 0.1 ml collected from each well for gamma counting. Input counts (32,994 counts per 5 min) were measured from 0.025 ml of labelled erthrocytes at 5×10^7 ml^{-1}. Final concentration of supernatant at the first dilution was 1/3. *b*, Complement-dependent lysis of DA lymphocytes assayed by release of ^{51}Cr. Viable lymph node lymphocytes were separated from erythrocytes and dead cells by centrifugation over Ficoll–Isopaque[6]. Chromium labelling and assay were as described for erythrocytes except that both procedures were performed at room temperature, labelled lymphocytes were diluted to 2.5×10^6 per ml, and the assay was extended to 90 min. Input counts (23,261 counts per 5 min) were measured from 0.025 ml of labelled lymphocytes at 2.5×10^6 per ml. (●), Titration of (AO × HO)F_1 anti-DA serum against DA lymphocytes. This serum lyses 100% of DA lymphocytes by Trypan blue exclusion at the dilutions studied in the conditions of this assay. Final concentration of supernatant at the first dilution was 1/3. *c*, Binding of R3/13 clone products to DA erythrocytes. 1.5×10^6 fresh washed erythrocytes were incubated for 15 min on ice with serial dilutions of supernatants in V-bottomed microtitre trays in a total volume of 0.05 ml. Cells were then washed twice in 0.2 ml PBS and resuspended with 0.01 ml (180,547 counts per 5 min) ^{125}I-rabbit F(ab')$_2$ anti-rat Fab (from Dr A. F. Williams). After a further 15 min on ice, cells were washed five times in 0.2 ml PBS and collected for gamma counting. Final concentration of supernatant at the first dilution was 1/2. Cell hybrids were prepared as follows: AO strain female rats were hyperimmunised by combined i.p. and subcutaneous immunisations with pooled spleen and lymph node cells from DA strain donors at monthly intervals (approximately 1/3 donor equivalent per recipient). Two and a half days before collecting spleen cells for hybridisation, hyperimmune AO rats received a final i.v. injection of DA lymphoid cells. Spleen cells for fusion were initially prepared in Dulbecco's PBS containing 2% FCS. The cells were then washed with Dulbecco's modified medium in the absence of serum (medium D), and 10^8 spleen cells mixed with 10^7 P3-X63-Ag8 cells[1] suspended in medium D' The mixture was centrifuged in a 50-ml plastic conical tube at 600*g* for 7 min. The supernatant was removed and the cell pellet disrupted by gently tapping the bottom of the tube. Further operations were performed at about 37 °C. A 1-ml pipette containing 0.8 ml of 50% PEG 1500 in medium D (*p*H 7.6–7.8) was used to suspend the cells gently while the solution was added over a period of 1 min. The suspension was kept at 37 °C for 1 min and 1 ml medium D added over a period of another min. A further 20 ml of medium D was added over a period of 5 min. The cells were centrifuged and gently resuspended in Dulbecco's modified medium containing 20% foetal calf serum (DMM). The suspension was distributed in 48 + 2 ml wells in Linbro BCL-5041 trays. In some experiments 3 + 10^5 untreated spleen cells were also added to each well to serve as a feeder. After 24 h half of the medium was replaced with HAT[8] medium. This operation was repeated in the two subsequent days and then every 2 d. Vigorous growth in a well after 14 d was taken as a successful hybrid clone(s). These were transferred into HAT medium lacking aminopterin for 4–7 days and thereafter into DMM.

(Ag-B5) cells, it was possible to show that the antigenic target for R3/13 antibody was specified in or near the chromosome region determining the major histocompatibility complex. The tests were performed on progeny from a breeding programme in which the Ag-B4 haplotype of DA is being backcrossed on to the HO background. Selected Ag-B5/Ag-B5 homozygotes and Ag-B5/Ag-B4 heterozygotes were used as target cell donors to assay the reactivity of positive R3/13 clone supernatants. The results of binding to erythrocytes and complement-dependent lysis of lymphocytes from these genetically typed donors show clearly that

Table 1 Strain distribution of antigenic target for R3/13 clone products

	Donor strain				
	AGUS	AO	DA	HO	
Input ^{51}Cr Cp5min	18,671	19,698	24,122	18,003	
C' control	2.6	2.0	2.5	2.3	Lymphocyte targets*: ^{51}Cr release as % of input counts
AO anti-DA serum at 1/48	51.7	3.9	71.0	60.8	
at 1/3072	1.9	2.1	66.3	2.6	
R3/13 clone 3 at 1/6	2.9	2.6	65.5	2.4	
6 at 1/6	2.6	2.5	60.4	2.1	
8 at 1/6	2.8	2.4	60.0	2.6	
9 at 1/6	2.9	2.9	62.7	2.7	
R3/13 clone 2 at 1/8	189	167	104	165	Erythrocyte targets†: ^{125}I rabbit F(ab')$_2$ anti-rat Fab Cp5min bound. Input cp5min = 100712
3 at 1/8	181	210	3,613	208	
6 at 1/8	128	149	3,518	176	
8 at 1/8	240	180	4,027	216	
9 at 1/8	158	174	3,882	166	
10 at 1/8	248	198	4,690	188	
14 at 1/8	144	324	3,995	119	

* Complement-dependent lysis of lymph node lymphocytes from AGUS (Ag-B1), AO (Ag-B2) (syngeneic control), DA (Ag-B4) and HO (Ag-B5) donors prepared and labelled with ^{51}Cr-NaCrO$_4$ as in Fig. 1*a*, *b*. Data extracted from full titrations of supernatants and serum performed as in Fig. 1. C' control contained DMM (1/6) instead of spent medium. AO anti-DA serum cross-reacts with AGUS and HO.

† Binding of clone supernatants to erythrocytes from AGUS, AO, DA and HO donors assayed by the second stage binding of ^{125}I-rabbit F(ab')$_2$ anti-rat Fab as in Fig. 1*c*.

the antigenic target of the R3/13 clone product is specified by a gene closely linked to Ag-B (Fig. 3).

The antigenic target of clone R3/13 is present on erythrocytes and on all lymphocytes of the DA strain. Furthermore, it has been shown (unpublished results) to be inherited with the main Ag-B4 serological specificities in the only described rat MHC recombinant[7]. These observations and the restricted strain distribution suggest that the R3/13 clone product recognises a K/D-like private specificity of the Ag-B4 haplotype. We presume that analysis of further clone products will enable other specificities of the Ag-B4 haplotype to be identified.

A remarkable feature of the lymphocytotoxic results in the linkage experiment (Fig. 3) was that the R3/13 clone product released only about 45% as much ^{51}Cr from the Ag-B5/Ag-B4 heterozygous cells and from the (HO × DA)F_1 as from cells of the DA strain, with a marked plateau at this level, suggesting that the target specificity in the Ag-B4 haplotype is weakly expressed, if at all, in about half the lymphocytes from a heterozygote. This phenomenon is not seen with an immune serum (unpublished results). Whether there is total exclusion of one allele for this individual specificity, or whether its expression is being modulated by other elements remains to be seen.

The results described here extend the use of fusion techniques for the derivation of permanent lines producing homogeneous antibodies to the increasingly important area of transplantation antigens. It is hoped that such antibodies will be invaluable in the precise definition of the rat MHC, in study of the expression of its components, and in analysis of functional properties of alloantibodies such as, for example, their role in enhancement. The antigenic target of clone R3/13 is the first uniquely defined antigenic specificity in the MHC. We suggest that this approach should eventually provide the basis for a standard MHC nomenclature. The derivation of a rat–mouse hybrid cell line producing antibody against a rat transplantation antigen brings the goal of producing standard permanent supplies of monoclonal antibodies for human tissue typing and other clinical uses one step nearer.

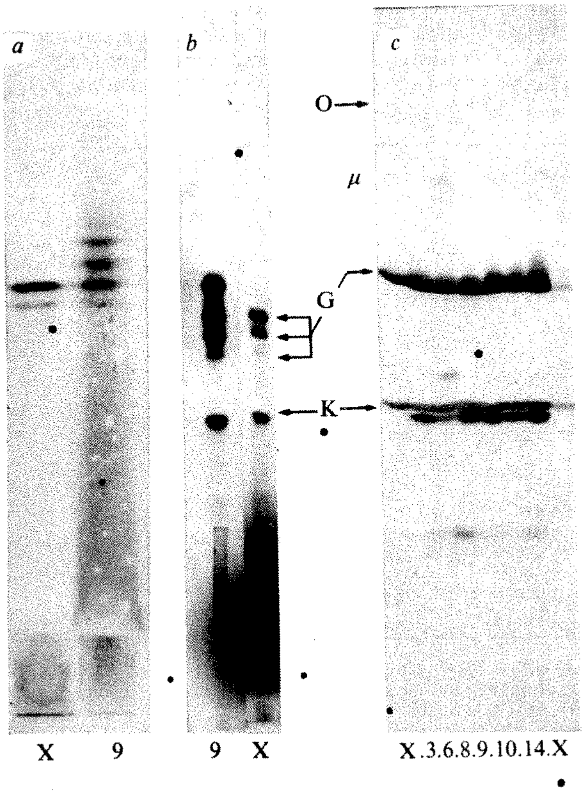

Fig. 2 Analysis of the products secreted by isolated plasma cell hybrid clones from R3/13. Cells were incubated with ^{14}C-lysine and the supernatants analysed as before[2]. *a*, Isoelectric focusing (*p*H 5, bottom; *p*H 9, top). *b*, Isoelectric focusing after reduction to separate the chains. *c*, SDS–polyacrylamide gel (10%) electrophoresis after total reduction. X refers to X63 control supernatants. Numbers refer to clones from culture R3/13 (see Fig. 1). O, origin; G and K, γ and κ chains from the Ig secreted by X63 cells; μ, putative μ chain referred to in the text.

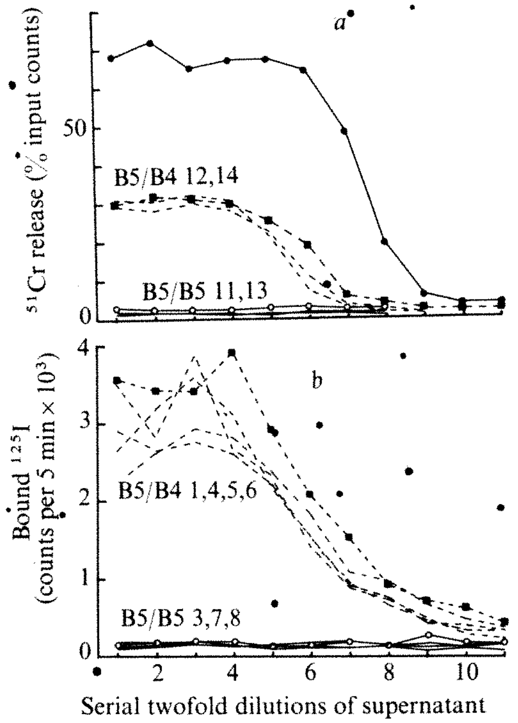

Fig. 3 Linkage of antigenic target of R3/13 clone products to Ag-B. Individuals from progeny of the fifth backcross of the Ag-B4 of DA onto the HO (Ag-B5) background were typed as either Ag-B5/Ag-B4 heterozygotes or Ag-B5/Ag-B5 homozygotes by conventional procedures[7]. Cells from typed individuals were then tested in two assays for the activity of the R3/13 clone product. *a*, Complement-dependent lysis of lymph node lymphocytes by R3/13 supernatant, assayed by release of ^{51}Cr. Lymphocytes were prepared, labelled and assayed as for Fig. 1*b*. Cells from DA (●), (HO × DA)F_1 (■) and the Ag-B5/Ag-B4 heterozygotes 12 and 14 (– – –) were lysed, while cells from HO (○) and the Ag-B5/Ag-B5 homozygotes 11 and 13 (—) were unaffected. Input ^{51}Cr counts for the seven populations tested ranged from 23,066 to 30,521 counts per 5 min. Final concentration of supernatant at the first dilution was 1/12. *b*, Binding of R3/13-9 clone product to erythrocytes. The binding of clone product was assayed indirectly as for Fig. 1*c*, by the subsequent binding of ^{125}I-rabbit F(ab′)$_2$ anti-rat Fab. Supernatant was bound to erythrocytes from the (HO × DA)F_1 (■) and the Ag-B5/Ag-B4 heterozygotes 1, 4, 5 and 6 (– – –) but not to erythrocytes from the HO (○) and the Ag-B5/Ag-B5 homozygotes 3, 7 and 8 (—). Input ^{125}I counts were 113,077 counts per 5 min. Final concentration of supernatant at the first dilution was 1/4.

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Neurite induction in mature cortical neurones in feline G_{M1}-ganglioside storage disease

PYRAMIDAL neurones of the human cerebral cortex develop spine-bearing structures (meganeurites) between the cell body and the initial portion of the axon in lysosomal storage diseases characterised by intraneuronal accumulation of gangliosides[1]. Meganeurites attain enormous proportions and acquire bizarre shapes, suggesting that their development involves restricted reactivation of embryonic growth mechanisms of the neurone. Because outgrowth of neurites is a sign of differentiation in embryonic neurones we have sought evidence for neurite outgrowth from meganeurites in feline G_{M1}-ganglioside storage disease[2–4]. We report here that in this system meganeurites of mature cortical neurones regularly exhibit extensive secondary outgrowth of neurites.

Brain tissue was obtained from an 11-month-old mutant cat with confirmed β-galactosidase deficiency and abnormally high levels of the monosialoganglioside G_{M1}-ganglioside in the brain[2]. The cat was killed with pentobarbital sodium near the terminal stage of the disease. This enabled us to obtain well preserved neurones and their processes for Golgi studies[1]. The brain was then perfused with a mixture of glutaraldehyde and paraformaldehyde for morphological studies.

Aberrant growth processes were observed in several varieties of cortical neurone. Most small and medium-sized pyramidal neurones of the cerebral neocortex had large pleomorphic meganeurites between the base of the perikaryon and the initial axonal region, as in human G_{M2}-gangliosidosis[1]. But the meganeurites in the feline G_{M1}-gangliosidosis had morphological features rarely observed in the human condition, in particular many fine neurites arising from them. These outgrowths had morphological features related to the cell type and characteristics of the relevant meganeurite (Fig. 1).

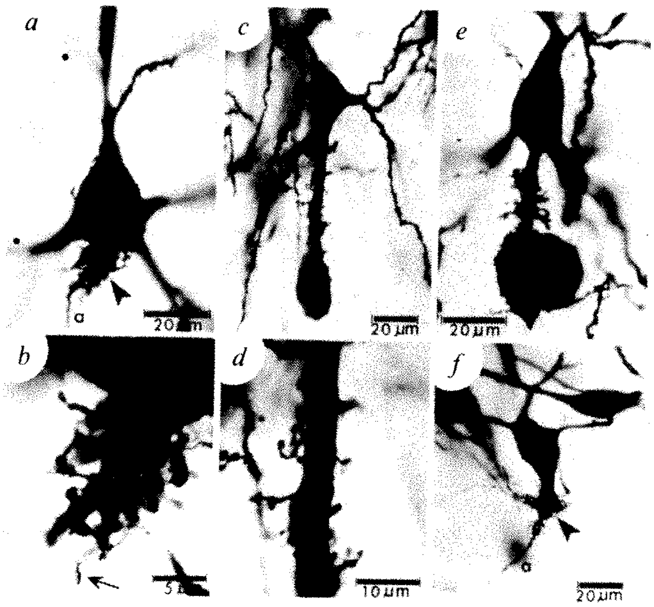

Fig. 1 Rapid Golgi preparations of cortical neurones in feline G_{M1}-gangliosidosis. *a*, Medium-sized pyramidal cell of the entorhinal cortex, meganeurite identified, at arrowhead. *b*, Enlargements of meganeurite in (*a*) to show tangle of neurites one of which has an expanded terminal head (at arrow). *c*, A neurone with a long meganeurite that gives rise to multiple neuritic growth processes, seen at higher magnification in (*d*). *e*, Another small pyramidal neurone of the cerebral cortex has a meganeurite that ends in a huge globular process. Outgrowth of neurites is prominent in the connecting stalk of the meganeurite. *f*, Granule cell of the fascia dentata of the hippocampus. Meganeurite, at arrowhead, exhibits extensive neuritic outgrowth (a, axon).

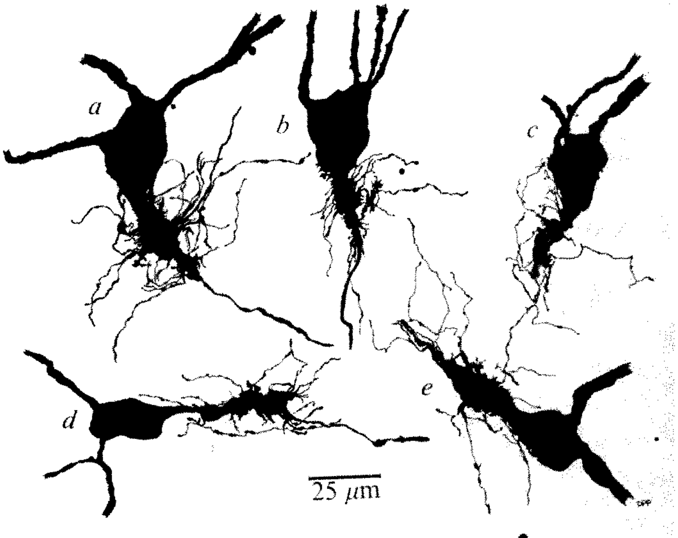

Fig. 2 Camera lucida drawings of granule cells of the fascia dentata. Only the proximal dendrites of these elements are illustrated. Cell bodies show variable shape distortion. Meganeurites arise from the base of the cell body and displace the axon distally. Extensive neurite proliferation and other growth processes are evident. Axons are identifiable in cells (*a*), (*b*) and (*d*). Fine processes also occur on portions of the cell bodies of neurones (*b*), (*c*) and (*e*).

A pyramidal neurone of the entorhinal cortex is shown in Fig. 1*a* with a meganeurite emerging from the base of the cell body. Many neuritic processes are shown as tangled outgrowths from the meganeurite, often with expanded terminal heads (Fig. 1*b*). A wide variety of neurites and other growth processes (spinules, spicules, filopodia-like elements) occur on larger meganeurites of small pyramidal neurones (Fig. 1*c*, *d* and *e*).

Extensive development of neurites on meganeurites was particularly clear in granule cells of the fascia dentata. In this relatively homogeneous population of small neurones, meganeurites formed large fusiform extensions from the base of the cell body, which is normally devoid of processes other than an axon. Long thin neurites appeared as tangled outgrowths from the meganeurite (Fig. 1*f*). The 'medusa-like' characteristics of these outgrowths is illustrated in camera lucida drawings of fascia dentata granule neurones (Fig. 2). In each of these elements the meganeurite is the site of extensive secondary neurite production. Neurites exhibit beading and tufted expansions, which are common features of neuronal growth processes in Golgi preparations[5].

G_{M1}-gangliosidosis is associated with the development of meganeurites on many types of cortical neurones in the cat, as is G_{M2}-gangliosidosis in man. Although secondary neurite formation has rarely been observed on meganeurites in classical Tay–Sachs disease[1] (infantile G_{M2}-gangliosidosis, B variant) accumulation of G_{M1}-ganglioside in feline cortical neurones seems to be a powerful stimulus for the outgrowth of secondary neurites from meganeurites in the feline mutant. Golgi studies of neurones in human G_{M1}-gangliosidosis will be necessary to determine whether the neurite proliferation is uniquely related to G_{M1}-ganglioside accumulation or the specific response of feline neurones to the ganglioside.

The demonstration that meganeurites in feline G_{M1}-gangliosidosis have growth characteristics typical of differentiating embryonic neurones raises the question of the role of gangliosides in neuronal development and synaptogenesis. Their distribution and content in the brain change during development and they are found in high concentrations in synaptosomes[6]. Although butyrate-induced process formation in differentiating HeLa cells is associated with an increase in G_{M3}-ganglioside[6], previous studies have not suggested specific involvement of gangliosides in neurite induction in normal embryonic neurones. Our results link G_{M1}-ganglioside accumulation to neurite formation in well-differentiated cortical neurones in an inherited metabolic disorder. It will be interesting to determine whether gangliosides induce or facilitate process formation in normal immature mammalian cortical neurones *in vitro*.

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The neural representation of visual space

THE approximate form of the projection of visual space on the striate cortex in man has long been established from neurological evidence[1–3] and estimates of cortical magnification M (the extent of striate cortex in millimetres corresponding to a degree of arc in visual space) have been derived from studies on cortical phosphenes and visual acuity[4], and migraine scotoma dimensions[5]. The possibility that M could be estimated from the density of retinal ganglion cells which provide the output from the eye to the brain has received support from studies on monkeys[6–8]. It has been shown that M is proportional to $\sqrt{D_c}$ (where D_c is the projected ganglion cell density in cells per solid degree of visual space) for peripheral angles (θ) greater than 10°. More centrally, where D_c is maximal, this relationship breaks down because the cells are displaced from their receptive fields by an amount which is difficult to determine[8]. If data on ganglion cell receptive field density, D_r (in receptive fields per solid degree) were available, they might be expected to relate to M at every point in the visual field. I report here that I have obtained such estimates of D_r and examined their usefulness as predictors of M. The results are summarised in three basic equations.

In previous studies on human retinal ganglion cell density it was assumed that solid angles were directly related to retinal areas[9–11]. Subsequently, it was reported that even at retinal level the projection of the visual field is progressively compressed towards the periphery[12]. Errors in estimates of D_c, due to the outward projection of an area of retinal section, and its location in the field could easily approach 100%. To avoid these, the original data from three studies on twelve human eyes (refs 9–11) were corrected by use of a specially designed schematic eye[12] which also diminished the errors arising from tissue shrinkage and sampling variations in ocular dimensions. The reprocessed values of cell density expressed in the form $1/\sqrt{D_c}$, plotted against θ, are shown in Fig. 1. This mode of display, originally popularised by Weymouth[13] is crucial to the argument by which the values of $1/\sqrt{D_c}$ are transformed to values of $1/\sqrt{D_r}$. As M is believed to be proportional to D_r the receptive field curve affords the possibility of estimating M at any peripheral angle θ. This relationship can, however, be further evaluated by correlating the $1/\sqrt{D_r}$ values with $1/M$ derived from studies of cortical phosphenes, visual acuity and migraine scotomata.

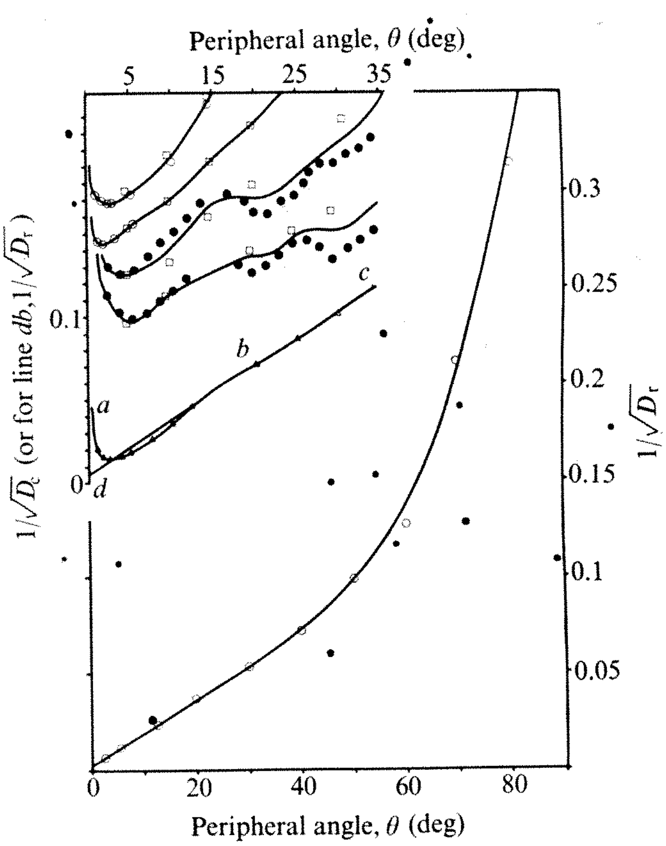

Fig. 1 The curve *abc* relates the value $1/\sqrt{D_c}$ (where D_c is the number of ganglion cells per solid degree) for the peripheral angle θ. It is the mean of the curves for the temporal nasal inferior and superior meridians of the visual field, sections of which are shown displaced upwards to avoid superimposition. The curves are based on the modified data (see text) of Vilter[9] ○, Van Buren[10] □, and Opel[11] ●. The curve *dbc* is similar but applies to receptive fields instead of ganglion cells. D_r is the receptive field density (per solid degree) and the curve relates $1/\sqrt{D_r}$ to θ. It was obtained by interpolating from *b*, beyond the area affected by excavation to *d*, at 0.0055 following Polyak's observations on the separation of the central-most foveal cones which he considered were equal in number to their ganglion cells[12]. It was realised that the two curves should determine equal populations of cells and numerical integration of D_c and D_r with solid angle showed only 6% difference in cell counts. The populations could be equated by slightly reducing the intercept value but conversely it would need to be slightly increased to take account of the findings of Missotten of the ratio of 0.9:1 for ganglion cells to cones in the rod free area[15]. The initial value was, therefore, retained as the best compromise for the purpose of the regression curve. The lower right coordinates refer to the plotted points of the final $1/\sqrt{D_r}$ values. The fitted curve is the curve of equation (1) when $k = 0.0055$. Integration of D_r with solid angle yielded an estimated total count of 1.5×10^6 receptive fields per eye.

The minimum angles of resolution obtained from the mean values of Wertheim's data on visual acuity[16], for horizontal and vertical meridians at eight selected eccentricities were compared with the receptive field data at the same points (Fig. 2). The points were chosen to give an appropriate weighting for the numbers of receptive fields within 40° peripheral angle which included more than 85% of the total count. The correlation coefficient with $1/\sqrt{D_r}$ values was very high ($r = 0.9934$) confirming some earlier suggestions[13,17]. The same approach was used on the data of Frisén and Glansholm for the horizontal meridian, which were obtained by a method which reduces optical degradation[18]. Surprisingly, the correlation was not quite so high, a fact which might be attributed to their use of a constant size of task field (4°) as Wertheim had enlarged the task details and field size equally.

The migraine scotoma has a characteristic angular velocity[19] and segment size[5,20] which varies with peripheral angle and is related to cortical magnification. Data were extracted from a graphical display[5] of scotoma dimensions and the least squares regression line predicting scotoma bar length from peripheral angle was used to derive best estimates for comparison with the $1/\sqrt{D_r}$ values. The correlation coefficient was again very high ($r = 0.9995$).

The most direct evidence of human cortical magnification was derived from the graphically displayed findings of Brindley and Lewin on visual field phosphenes resulting from cortical stimulation[21]. The angular separation of phosphenes was divided by the linear separation of electrodes to yield a value inversely proportional to cortical magnification. The method of obtaining these values, though completed independently, resembled that of Cowey and Rolls[4]. A regression line again provided the best estimate of the reciprocal of cortical magnification for selected values of θ which were correlated ($r = 0.9992$) with the $1/\sqrt{D_r}$ values (see Fig. 2).

These comparisons clearly show that $1/M$ is almost exactly proportional to $1/\sqrt{D_r}$. Evidently therefore M^2, the cortical projection of a solid degree is proportional to D_r the number of receptive fields per solid degree, and the constant which relates them must be the total striate area (for which 6,000 mm² would be an acceptable estimate[4]), divided by 1.5×10^6, the total ganglion cells of one eye (Fig. 1 legend). For the data of this study therefore, $M^2 = 0.004\ D_r$ and since the central foveal value of $1/\sqrt{D_r}$ was found to be 0.0055, D_r must be 33,058 cells per solid degree so that $M^2 = 132$ mm² per solid degree, and $M = 11.5$ mm per degree. This maximal value of M is close to that suggested by Richards[5], but differs substantially from the 15.6 mm per degree found by Cowey and Rolls[4]. It has been found, however, that the discrepancy diminishes peripherally and the results are not statistically incompatible. Cowey and Rolls used the M values of Brindley and Lewin, and related these to the acuity data of Wertheim to obtain their result. This depended in the similarity of the visual characteristics of two individual subjects. The finding of the present report is clearly based on extensive new data, and probably therefore more reliable.

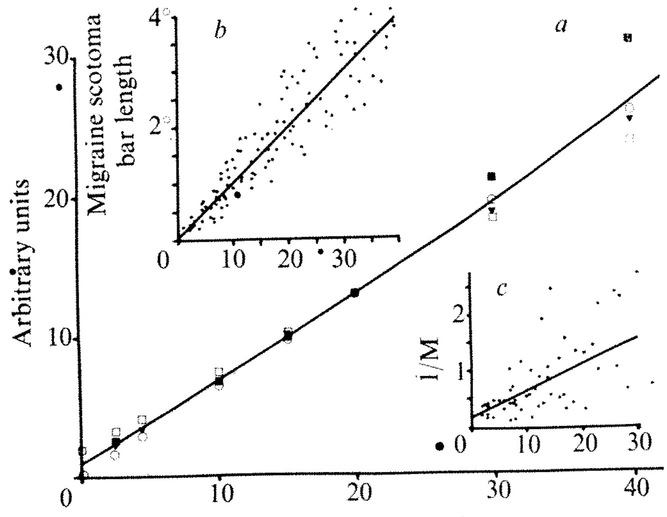

Fig. 2 *a*, Receptive field data (▼) and curve plotted for comparison with minimum angle of resolution data (■), best estimates of migraine scotoma size (○) and cortical magnification (□). All data are scaled to coincide at 20° where the ganglion cells are unaffected by excavation and where the best estimates of migraine scotoma lengths and cortical phosphene M values are most reliable. Visual resolution at this point is also considered to be relatively unaffected by optical degradation. The origin of the best estimates is shown in (*b*) and (*c*) which are based on the data of Richards[5] and Brindley and Lewin[21]. The main ordinate is scaled in arbitrary units based on the intercept of the receptive field curve which therefore satisfies equation (1) when $k = 1$. The displacement of the receptive field data points relative to the curve as compared with Fig. 1 is due to the rescaling at the 20° point. The graph shows the proportionality between the different criteria of cortical representation which are reflected in the high correlation coefficients quoted in the text.

Due to the near-perfect correlations between the $1/\sqrt{D_r}$ values and other criteria, the following equation not only describes the variation of $1/\sqrt{D_r}$ with θ, but has some remarkably general properties in relation to the structure and functions of the visual system

$$V=k[1+S\theta(1+3\theta^2\times10^{-5}+8(S\theta)^{5.5}\times10^{-10})] \qquad (1)$$

V provides an estimate of $1/\sqrt{D_r}$ or any of its correlates, such as $1/M$ or the threshold of resolution, at a peripheral angle θ if in each case the foveal value is substituted for k. S determines the initial slope of the regression curve but the peripheral slope and the limit of the visual field are controlled by the last two terms. If data averaged for all meridians are considered, $S = 0.59$ and the linear relationship, $V = k(1+0.59\theta)$ gives a useful approximation when θ is less than 30°.

The number of receptive fields per solid degree at a peripheral angle θ is easily estimated from equation (1). If $k = 0.0055$ the $1/\sqrt{D_r}$ value at the fovea, V gives $1/\sqrt{D_r}$ at θ from which D_r is deduced. Similarly, if $k = 1/M$ for the fovea, or 1/11.5 then $V = 1/M$ at $\theta°$, and M, and thus M^2 are easily obtained. (In the case of the monocular temporal crescent, however, the estimates of M and M^2 should be divided by $\sqrt{2}$ and 2, respectively.) Again, if k is the foveal threshold of resolution in minutes of arc, V provides the value at θ and thus its reciprocal, the Snellen decimal acuity. The maximum spacial information density in bits per solid degree is then $3600/V^2$, following the logic of simple information models of vision[22]. Such estimates are, of course, primarily related to the threshold of practiced observers.

The use of equation (1) is extended specifically to the temporal nasal, superior and inferior portions of the principal meridians of the visual field, if $S = (0.46-0.00043\theta)$, $(0.50+0.0019\theta)$, $(0.62+0.0033\theta)$ or $(0.66-0.0006\theta)$. The corresponding curves, as with the general form of equation (1), have a short portion which is adequately simulated by a straight line. In this case $S = 0.46$, 0.50, 0.62 or 0.66 and $V = k\ (1+S\theta)$. This simple relationship is applicable when $\theta <$ 35°, 20°, 10°, and 20°, taking the meridians in the above stated order.

The linear function, $V = k(1+S\theta)$, for small angles permits the possibility of the following analytical solution to the problem of cumulative estimates:

$$C = \int_0^\theta \frac{2\pi\theta d\theta}{[k(1+S\theta)]^2} = \frac{2\pi}{(kS)^2}\left[\log_e(1+S\theta)+\frac{1}{(1+S\theta)}-1\right] \qquad (2)$$

This gives a cumulative estimate which is within 1% of the value indicated by this study if $\theta <$ 10°, thereafter it becomes less accurate and overestimates by 5% at 45°. The symbols are as in equation (1), thus if K is 0.0055 equation (2) gives an estimate of the total number of receptive fields in a concentric solid angle of visual space, where θ is the angular semi-diameter. Similarly, if $k = 1/M$ for the fovea, that is 1/11.5, equation (2) gives the area of cortex (mm²) again corresponding to the same concentric solid angle. Due to the nature of the cortical projection, however, areas of less than 100 mm ² are best described as a percentage of cortical input.

When the receptive field counts assessed by numerical integration of D_r and solid angle were examined it was noted that the cumulative count can be described by the simplest and perhaps the most useful equation

$$Q = 100[1-\exp(-0.0574\theta)] \qquad (3)$$

where Q gives a close approximation to the percentage of total receptive fields, striate area or information channel

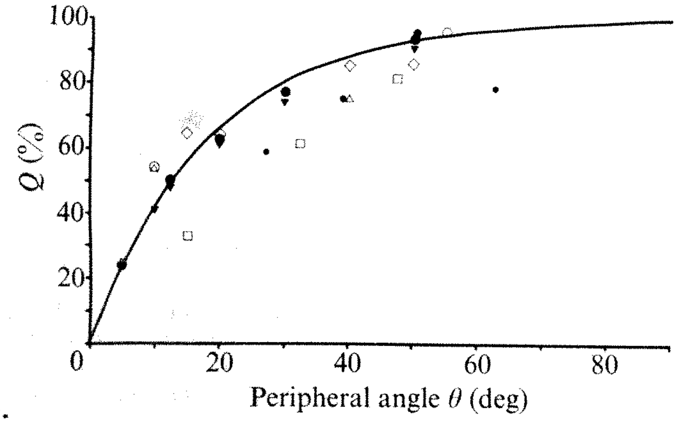

Fig. 3 Cumulative counts of ganglion cell receptive fields provided a method of estimating the percentage of the surface area of the striate cortex corresponding to a concentric region of visual space. Q is cumulative % of receptive fields, cortical area or channel capacity; ●, % receptive fields found in this study; ▼, % using equation (1) instead of actual data, curve is that of equation (3). Additional points derived from the estimates of areas from neurological maps of Holmes[1] □, Spalding[2] △, Teuber *et al.*[3] ◇, and Duke Elder[23] ○ are shown for comparison. Although such charts are designed to convey a quantitive impression, ambiguity is inevitable with the two-dimensional projection of the brain surface. Approximate values were obtained by studying the charts in conjunction with anatomical material and models, and assessing the area of redrawn sections by use of rectangular grids.

capacity for a concentric solid angle of visual space with angular semi-diameter of θ°. The cumulative count and the curve of equation (3) are shown in Fig. 3 and these may be compared with the result of an attempt to relate the present findings to accepted neurological schemes.

Contemporary theories present a complex picture of the neural connections of the visual system, but this does not diminish the usefulness of these equations which merely reflect the quantative relationships between assembled data and do not depend on a hypothesis involving sensory units.

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Enkephalin may mediate euphoria and drive-reduction reward

THE identification in brain of opiate receptors[1] and morphine-like pentapeptides (methionine-enkephalin and leucine-enkephalin)[2] has led to suggestions that enkephalin receptors may be sites at which opiate drugs induce analgesia and euphoria, and that the enkephalins themselves may be transmitters or modulators in brain systems for the regulation of pain and pleasure[3–6]. Observations in animals reveal that appropriate central administration of enkephalin produces morphine-like analgesia[7–9] and physical dependence[10]. We report here that enkephalin also may share the euphorigenic or rewarding properties of morphine in that rats will work for enkephalin injections delivered directly into the ventricles of their own brains[11]. Of particular interest, in the light of the generally superior potency of the methionine peptide[2,8,9], is the observation that leucine-enkephalin is self-administered with greater avidity than methionine-enkephalin.

Guide cannulae, of 19-gauge stainless steel tubing, were implanted above the lateral ventricle in 300–400-g male Charles River rats under pentobarbital anaesthesia. Following a recovery period of at least 1 week, rats were tested for intraventricular self-administration behaviour in a Skinner Box[12]. A 25-gauge stainless steel injection needle was inserted in the lateral ventricle via the guide cannula. The needle was connected by polyethylene tubing through a feedthrough swivel device to an infusion pump. The tubing contained one of the substances (dissolved in Ringer's solution and adjusted to pH 5.8) listed in Table 1. Each lever-press response delivered 1 µl of fluid to the brain in 0.9 s; however, responses made during the 0.9-s infusion intervals had no consequences and went unrecorded. Each rat was tested once and had access to only one solution. No lever-press training was given; rats were merely placed in the Skinner box, with food and water available, for a 66-h session.

The peptides were synthesised at Wyeth Laboratories or purchased from Bachem Inc. After purification by gel filtration (Sephadex G10), homogeneity of the peptides was verified by amino acid analysis and thin-layer chromatography. The use of highly purified material was essential; central administration of unfiltered leucine-enkephalin often caused lethal convulsions. After storage in polyethylene tubing for 66 h, solutions of methionine-enkephalin exhibited a negligible loss of activity in a standard opiate receptor binding assay[9].

Rates of self-administration were significantly higher for the enkephalins and morphine (but not the structurally related tetrapeptide, Tyr–Gly–Gly–Phe) than for Ringer's solution (Table 1). The order of preference for the peptides when tested at either 1 or 10 µg per injection was leucine-enkephalin > methionine-enkephalin > Tyr–Gly–Gly–Phe (Fig. 1). Both concentrations of leucine-enkephalin were self-injected at a higher rate than the single dose level of morphine (0.5 µg per injection), although in neither case was the difference statistically significant. The 1-µg doses of both pentapeptides caused more rapid learning of self-administration behaviour than the 10-µg doses, but the 10-µg doses generated a more sustained performance level (Table 1). Massive dosages of leucine-enkephalin (up to 10 mg in 66 h) were taken by the best responders, despite the possibility of tissue damage or other adverse effects that might have been produced by the passage of large volumes of fluid through the brain ventricles. Indeed, injections of the Ringer's vehicle itself suppressed lever-pressing behaviour. The mean response rate in the Ringer's group fell significantly below that of a non-injected group tested with empty syringes ($P < 0.005$).

To test that nonspecific behavioural stimulation was not

Table 1 Summary data of self-administration experiments

Drug	No. of rats	Dose (μg μl^{-1})	Mean self-administration responses ± s.e.m. First 24 h	Final 24 h	66-h Total	Highest 6-h rate
Ringer's	25		64.0±10.7	54.7±11.3	167.9±24.3	47.6±6.2
Morphine sulphate	9	0.5	103.4±35.2	89.1±14.4	283.0±67.1*	54.9±13.6
Tyr–Gly–Gly–Phe	5	1	71.8±29.5	96.8±47.7	237.6±103.0	51.0±18.3
	14	10	74.0±17.4	85.0±26.8	221.8±54.2	56.6±11.8
Tyr–Gly–Gly–Phe–Met	15	1	103.9±16.5†	120.7±41.9*	287.5±70.1*	68.7±13.4
	12	3.3	93.3±14.8	86.2±15.2	252.1±35.6*	56.8±9.6
	19	10	79.2±14.9	105.5±21.5†	266.6±50.7*	56.8±8.1
Tyr–Gly–Gly–Phe–Leu	6	1	141.7±29.8‡	99.7±36.8	333.2±60.9‡	83.3±12.0†
	18	10	116.6±43.2	186.9±56.4†	432.4±131.3†	110.3±28.2†

*Significantly different from Ringer's solution, $P<0.05$.
†$P<0.025$.
‡$P<0.005$.

Rats implanted with permanently-indwelling intraventricular cannulae had continuous access to one of the indicated drug solutions in a single 66-h test. Each lever-press response delivered 1 μl of solution into the lateral ventricle in 0.9 s.

responsible for the high response rates in the enkephalin groups, rats previously trained to lever press for food or brain-stimulation rewards were treated with leucine- or methionine-enkephalin (50–200 μg, intraventricularly) just before the behavioural test. Lever-press rates were suppressed rather than facilitated by both peptides (Table 2), and observations of the animals revealed that their activity level had been depressed rather than increased. The behaviour more plausibly may reflect a morphine-like rewarding or addicting action of the peptides. The observed preference for leucine-enkephalin over methionine-enkephalin also may be consistent with a reward interpretation. Opiate receptor binding by the leucine peptide is reduced more by sodium and enhanced more by manganese than is the case for the methionine peptide[9,13]. Since a similar sensitivity to sodium and manganese differentiates the highly addictive opiate agonists from other classes of opiate drugs[13] these observations provide independent evidence that leucine-enkephalin may be a 'purer' opiate agonist with greater addictive or euphorigenic potential than methionine-enkephalin.

Why would a natural substance such as leucine-enkephalin possess addictive properties? One interesting, if highly speculative, possibility is that a peptide similar or identical to leucine-enkephalin may serve as a natural euphorigen[6] or reward transmitter. If this conjecture were true, reward or addiction should also be produced by release of endogenous peptide following electrical activation of enkephalin-containing neurones in the brain. Behavioural and immunohistochemical observations may be consistent with this prediction. Sites that yield high rates of self-stimulation[14] and those that contain dense networks of enkephalin-like immunoreactivity[15] often overlap in precisely the same brain regions (for example, pontine central grey, zona compacta of the substantia nigra, bed nucleus of the stria terminalis and nucleus accumbens). According to our hypothesis, self-stimulation of these regions would depend at least in part on the electrically-induced release of enkephalin and the consequent activation of opiate 'reward' receptors. If so, the behaviour should be suppressed or extinguished following administration of an opiate receptor antagonist such as naloxone.

Fig. 1 Intraventricular self-administration of opiate peptides and morphine. *a*, 10 μg per injection; *b*, 1 μg per injection. Curves show mean number of self-injections, cumulated at successive 6-h intervals over the entire 66-h test, of different groups of rats. For further details see Table 1. ■, Tyr–Gly–Gly–Phe–Leu; ×, morphine SO$_4$ (0.5 μg); ●, Tyr–Gly–Gly–Phe–Met; ▲, Try–Gly–Gly–Phe; ○, Ringer's.

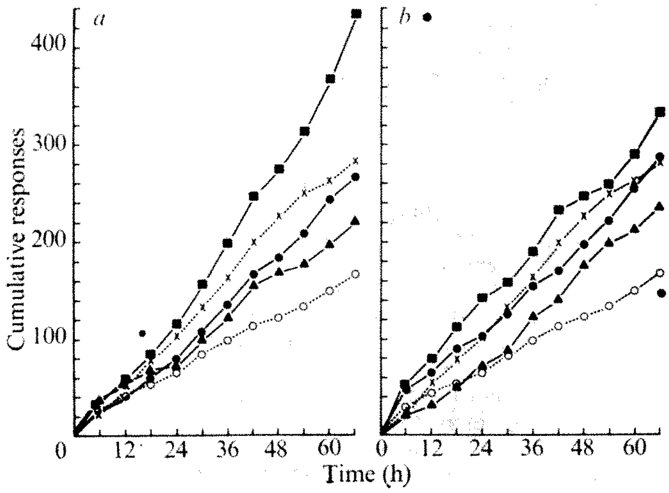

Table 2 Suppressant action of enkephalin on operant behaviour maintained by food or brain-stimulation reinforcement

Drug	Dose (μg)	Lever-press rate (% of no drug) Food	N	Brain stimulation	N
Ringer's		125.5±23.4	4	101.8±12.4	9
Methionine -enkephalin	50	57.6±25.6	4	not tested	
	100	46.1±35.9	4	84.3±5.2	3
	200	not tested		38.8±28.6	2
Leucine -enkephalin	100	57.8±16.1	8*	61.1±2.0	2
	200	39.8±39.5	2	28.6±0.8	2*

*Significantly different from Ringer's, $P<0.05$.

Lever-press responses were reinforced either by electrical stimulation of the medial forebrain bundle on a continuous schedule or by presentations of sweetened milk on an aperiodic schedule (average, once every 2 min). All drugs were dissolved in 10 μl of Ringer's solution and injected in the lateral ventricle either 20 min after the start of the 40-min brain-stimulation reward test or immediately before the 72-min food reward test. *N*, number of rats.

Eight rats with electrodes in or just lateral to the pontine central grey received self-stimulation training until their 1-h response rates had stabilised (range, 1,200–5,000 responses per h). The central grey region was selected for initial testing because electrical stimulation of this site induces profound analgesia as well as high-rate self-stimulation[16]. Naloxone hydrochloride (0.1–10 mg kg^{-1}, subcutaneously) was administered at weekly intervals immediately before the behavioural test. As predicted, dose-related decreases in the self-stimulation rate were obtained in virtually every case (Fig. 2). Because the central grey region contains high concentrations of noradrenaline[17], a substance previously shown to be involved in self-stimulation[18], the same rats were further tested with different doses of the noradrenaline synthesis inhibitor, diethyldithiocarbamate. Again,

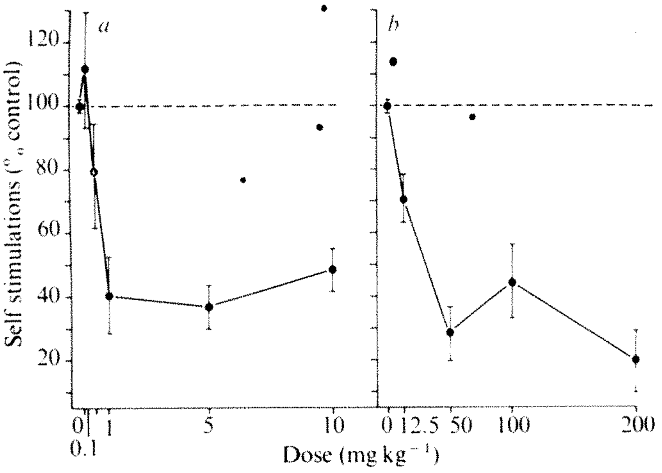

Fig. 2 Dose-related suppression of central grey self-stimulation following subcutaneous administration of the opiate antagonist naloxone (*a*) or the dopamine-β-hydroxylase inhibitor diethyldithiocarbamate (*b*). Each data point is the mean of 3–8 observations. Bars indicate standard errors.

response rates were suppressed in a dose-related fashion (Fig. 2). These results suggest that central grey self-stimulation may depend on the activation of both noradrenaline-containing and enkephalin-containing neurones; indeed, since all of the enkephalin-rich self-stimulation sites referred to above are also rich in catecholamines[17], it is possible that the reward process may generally be regulated by the interaction of noradrenaline, dopamine, and enkephalin systems. Studies on intravenous self-administration of opiate drugs similarly suggest that catecholamine mechanisms are involved in opiate reinforcement[19,20].

Diverse, but complementary, reward functions can also be recognised at the behavioural level. One of these corresponds to a state of incentive, and is the process by which behaviour in pursuit of a goal is motivated and steered; this 'drive-inducing' reward function may be mediated by catecholamines[21]. The second corresponds to a state of satisfaction or well being, and is the process by which an episode of behaviour is ended following the attainment (and consumption) of the goal; this 'drive-reducing' reward function may be mediated by an opiate peptide such as leucine-enkephalin.

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Selective vulnerability of neurones in organic dementia

THERE are many causes of the clinical state dementia. Excluding those patients with tumours, infection or vascular disease, there remains a large group[1] whose brains are atrophied and contain an excess of senile morphological change (senile plaques, neurofibrillary degeneration and granulovacuolar change) in the cortex. When this condition occurs before the age of 65 years it is known as presenile dementia or Alzheimer's disease; after this age it has been called senile dementia. Since there is no good reason on pathological grounds[2] to maintain this distinction, we use the term Alzheimer's disease irrespective of the patient's age. Together with Parkinson's disease and Huntington's chorea, Alzheimer's disease is one of a group of disorders that Gowers (see ref. 2) called abiotrophies, in which nerve cell loss appears to occur in characteristic patterns (that is, substantia nigra, basal ganglia and cortex, respectively). The cause of this cellular death remains unknown. Morphological assessment of possible nerve cell loss[2] or glial cell increase[3] has been difficult, particularly in Alzheimer's disease. We have sought therefore to complement histological findings by measuring appropriate biochemical constituents of brain as indices of total neural cell numbers. Whole temporal lobes have been homogenised and analysed and the results expressed per lobe. The results[4] suggest that whilst the total number of glial cells is relatively unchanged, there is a loss of approximately half the total nerve cell population. This estimate is based on comparison with age-matched controls, and so the selective neuronal loss is probably a disease-related phenomenon. These findings conform with conclusions drawn from morphological data[5].

Evidence of selective changes in the number or state of specific types of neurone has been obtained by measuring the concentration of putative neurotransmitters and their biosynthetic enzymes. Thus there is selective reduction in the concentrations of dopamine[6] in the substantia nigra and GABA[7] in the striatum in patients with Parkinson's disease and Huntington's chorea respectively, suggesting a defect in dopaminergic or GABA-containing neurones. Choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD) are believed to be markers of cholinergic and GABA-containing neurones, respectively[8]. In Huntington's chorea GAD activity is selectively depleted only in morphologically affected regions[9,10]. There are indications, however, that in Alzheimer's disease, the activity of CAT is reduced (compare groups 4 and 5, Table 1) and that this parallels increasing senile morphological change[8], which in turn correlates with dementia score[11].

It is unclear whether or not our earlier finding of reduced cortical GAD activity in Alzheimer's disease (group 5, Table 1) accurately reflects the pre-terminal state of GABA-containing neurones[8]. This is because most demented patients die from severe bronchopneumonia[8] and probably also have a defective respiratory reflex[12] with reduced cerebral blood flow terminally[13]. This is important, for GAD activity and the concentration of a soluble acidic brain protein (neuronin S-6) seem to decline both in baboon brain with reduced cerebral blood flow[14] and in human brains from non-demented patients either dying of bronchopneumonia (compare GAD activity in groups 3 and 4, Table 1) or with evidence of more severe terminal cerebral hypoxia[8]. Furthermore, the concentration of neuronin S-6 is markedly depleted in cortex from cases of Alzheimer's disease[8,15]. There are indications[16] that this decrease in GAD activity is because of denaturation at acidic *p*H values. Our findings, and those of McGeer and McGeer[10] on the effects of terminal coma, demonstrate the importance of making allowance for the agonal state.

In an attempt to resolve these difficulties we have examined cortical biopsies removed at craniotomy (group 2, Table 1). Most of these were cases of Alzheimer's disease, and the mean CAT activity is reduced by over 50% while GAD activity is

Table 1 Choline acetyltransferase (CAT) and glutamate decarboxylase (GAD) activities in human post-operation and post-mortem neocortical specimens

Brain specimen and group no.	CAT activity (units per min)	GAD activity (units per min)	Total protein (mg per g wet weight)
Post-operation specimens			
(1) Control tissue*	66.80±26.96 (10)	1333±467 (8)	81.2±17.5 (10)
(2) Alzheimer's disease and related disorders†	27.52±15.38 (6)[a]	1393±329 (5)	75.7±17.6 (6)
Post-mortem tissue‡			
(3) Controls dying suddenly	62.78±14.75 (12)	1090±460 (17)	74.6±14.9 (17)
(4) Controls dying of bronchopneumonia	71.83±16.42 (7)	690±182 (8)[c]	75.1±18.4 (7)
(5) Alzheimer's disease dying of bronchopneumonia	36.62±10.65 (6)[b]	295±89 (9)[d]	75.1±10.4 (8)

CAT, GAD and protein were measured as previously described[8]. Data are mean ± s.d.; numbers in brackets are number of cases; enzyme units are pmol acetylcholine or GABA formed per mg protein. [a]–[d]Identify significant differences between closely matched groups. CAT activity: [a]group 2 compared with 1, $P<0.01$; [b]group 5 compared with 4, $P<0.001$. GAD activity: [c]group 4 compared with 3, $P<0.05$; [d]group 5 compared with 4, $P<0.001$.

*Normal neocortex removed at surgery for tumours; mean age 42±20 (range 10–67). For these specimens neither CAT nor GAD are age-dependent which contrasts with data for post-mortem samples from cases aged 5–50 (ref. 23).

†Neocortex from 2 biopsy confirmed cases of Alzheimer's disease and 2 probable cases; an adolescent with cerebral atrophy and some symptoms of schizophrenia ('dementia praecox') and a case of Parkinson's disease with a sister with pre-senile dementia; mean age 49±18 (range 18–65).

‡Calculated from previously published data for neocortex from cases matched with respect to the interval between death and autopsy. The ages for groups 3, 4 and 5 are 72±13 (range 50–100), 66±16 (range 37–84) and 85±5 (range 79–97). For these specimens neither CAT nor GAD activities are age-dependent[8].

unaffected. For the four Alzheimer cases alone CAT activity (24.10±18.63 units) is reduced ($P<0.02$) by a mean of 64% while GAD activity (1298±205 units) is unchanged. The results for the biopsy samples are unique for this is the first time that CAT activity in diseased human brain has been shown to be reduced with GAD activity remaining unaltered. Comparable results have been reported recently[17] for the density of putative acetylcholine and GABA-receptor binding sites in striatum in Huntington's chorea.

The depletion in CAT activity that we have detected is of particular interest because the anticholinergic glycolate esters, of which the muscarinic cholinergic marker quinuclidinyl benzilate[17] is a prominent example, can cause complete amnesia[18]. Furthermore, despite inconsistencies in the literature[19], at least one pharmacological study[20] on humans indicates that the cholinergic system is involved in age-related memory degeneration. In order to provide a rational basis of treatment other tentative markers of the state and number of specific types of neurones are being measured. Preliminary results are encouraging for in one case (a 63-yr-old patient that probably had Alzheimer's disease) CAT activity in the biopsy was reduced by 67% and the high affinity uptake of choline was found to be reduced by 66% (the control uptake data ±s.d. expressed as c.p.m. per mg protein per c.p.m. per ml medium[14] being 0.0097±0.0029, $N=7$). In contrast, GAD activity and guanylate and adenylate cyclase activities[16] (the latter being potential indices of the post-synapse) and β-alanine independent GABA uptake[14] were all within 1.5 s.d. of the appropriate mean control value.

When the data for the biopsy and autopsy samples are considered together they suggest that at least the presynaptic cholinergic system is selectively affected in Alzheimer's disease. This perhaps offers the possibility of treatment with a centrally acting anticholinesterase for at present only about 14% of cases of organic dementia are treatable[21]. This therapeutic approach may not be entirely successful, however, as there are indications that other aspects of brain metabolism may be decreased, which may interact with the presynaptic cholinergic system[22].

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Note added in proof: Data published recently[24–27] substantiate our contention that the pre-synaptic part of the cholinergic system might be selectively affected in Alzheimer's disease.

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Catechol oestrogens inhibit oestrogen elicited accumulation of hypothalamic cyclic AMP suggesting role as endogenous anti-oestrogens

THE catechol oestrogens, including 2-hydroxyoestradiol and 2-hydroxyoestrone, are recognised as major metabolic products of oestrogen[1]. Catechol oestrogens are present in both pituitary and hypothalamus in concentrations at least tenfold higher than the parent oestrogens[2]. In addition, the synthesis of catechol oestrogens by rat hypothalamic tissue *in vitro* has also been reported[2,3]. Catechol oestrogens compete with 17-β-oestradiol (E_2) for soluble oestrogen receptors of the pituitary and hypothalamus[4], suggesting a role for these compounds in the control of gonadotrophin secretion within the pituitary–hypothalamic axis. We have investigated the effects of 2-hydroxyoestradiol on the oestrogen-elicited accumulation of cyclic AMP in intact hypothalami *in vitro*, and we report here that 2-hydroxyoestradiol antagonises the increase in cyclic AMP elicited in hypothalami of immature female rats by both diethylstilboestrol (DES) and E_2, suggesting that 2-hydroxyoestradiol may function as an endogenous anti-oestrogen.

Confirming previous reports[5,6,12,13], both DES and E_2 significantly stimulated the accumulation of cyclic AMP in incubated rat hypothalami. Preincubation of hypothalami with equimolar (20 μM) concentrations of 2-hydroxyoestradiol significantly inhibited the DES and E_2 stimulated accumulation of cyclic AMP by 57% and 68%, respectively (Table 1). At 20 μM, 2-hydroxyoestradiol alone did not significantly stimulate the accumulation of cyclic AMP. In contrast to inhibitory effects of 2-hydroxyoestradiol, its O-methylated metabolite (2-methoxyoestradiol) was inactive at concentrations up to 50 μM. Previous studies[5] have shown oestrone and oestriol to have no significant stimulatory effects on the accumulation of cyclic AMP. Equimolar (20 μM) concentrations of either oestrone or oestriol inhibited DES-elicited accumulation of cyclic AMP only marginally. The anti-oestrogen clomiphene citrate (20 μM) was previously shown to antagonise[5] (by 50%) the effects of DES (20 μM); this finding was confirmed in the present study (41% inhibition, see Table 1).

The ability of oestrogenic compounds to stimulate the formation of cyclic AMP in the isolated hypothalamic preparation seems to parallel the oestrogenic potency of these compounds both *in vivo* and *in vitro*[5,6]. In addition, the oestrogen-elicited increases in cyclic AMP are antagonised by the anti-oestrogen clomiphene and are not observed with physiologically inactive oestrogen enantiomers such as 17-α-oestradiol. Furthermore, both clomiphene and 2-hydroxyoestradiol are ineffective in antagonising the effects of DES if added 40 min after the oestrogen, and both α- and β-adrenergic antagonists are effective inhibitors[6,12]. These observations suggested that an initial binding to hypothalamic oestrogen receptors is a prerequisite for DES- or E_2-elicited accumulation of cyclic AMP and the stimulatory effects of oestrogens on cyclic AMP formation result from an oestrogen-receptor dependent release catecholamines rather than a direct activation of an oestrogen-receptor linked adenylate cyclase[6].

Our data clearly demonstrate that the catechol oestrogen 2-hydroxyoestradiol, like clomiphene, antagonises the effects of both DES and E_2 on generation of cyclic AMP in the hypothalamus, whereas weakly oestrogenic compounds such as oestriol and oestrone are ineffective as either agonists[5,6] or antagonists. The observation that 2-methoxyoestradiol does not antagonise the effects of DES *in vitro* indicates that *O*-methylation may serve as a major route of inactivation for these compounds as this pathway proceeds readily both *in vitro*[14] and *in vivo*[15].

The presence of catechol oestrogens in brain and various endocrine tissues in concentrations that exceed those of the parent compounds[2] suggests that these catechols could function as endogenous anti-oestrogens, and thus may regulate the actions of oestrogens *in situ*. Reports that administration of 2-hydroxyoestrone to ovarectomised, oestrogen-primed female rats[16] or immature male rats[17]

Table 1 Inhibition of oestrogen-elicited accumulation of hypothalamic cyclic AMP by 2-hydroxyoestradiol

	Additions	Cyclic AMP (pmol per mg protein)	Inhibition (%)
Series I			
	None	8.9±0.9 (8)	
	DES (20 μM)	25.5±2.4 (7)	
	2-hydroxyoestradiol (20 μM)	9.0±2.6 (3)	
	DES (20 μM) + 2-hydroxyoestradiol (20 μM)	15.9±2.2 (6)	57*
	DES (20 μM) + 2-methoxyoestradiol (50 μM)	25.5±1.5 (3)	0
	17-β-oestradiol (20 μM)	12.0±0.5 (5)	
	17-β-oestradiol (20 μM) + 2-hydroxyoestradiol (20 μM)	9.9±0.9 (4)	68†
Series II	Additions	Cyclic AMP (pmol per mg protein)	Inhibition (%)
	None	6.3±0.6 (7)	
	DES (20 μM)	18.4±0.5 (6)	
	DES (20 μM) + clomiphene citrate (20 μM)	13.4±1.4 (5)	41§
	DES (20 μM) + 2-hydroxyoestradiol (20 μM)‡	16.8±1.5 (6)	14
	DES (20 μM) + oestrone (20 μM)	16.3±1.8 (7)	17
	DES (20 μM) + oestriol (20 μM)	18.6±1.4 (6)	0

Female Sprague–Dawley rats (Taconic Farms), aged 26–28 d were used. Animals were killed by decapitation and hypothalami removed and dissected[5,6]. Hypothalami from groups of 10 rats were incubated at 37 °C in 20 ml Krebs–Ringer bicarbonate buffer with glucose (KRBG)[7] and gassed with 95% O_2–5% CO_2. After 45 min hypothalami were washed once with an equal volume of KRBG and transferred to individual 30-ml beakers containing 10 ml of KRBG plus test agents. Steroids were dissolved in 75% ethanol and added in a volume of 20 μl. Hypothalami were pre-incubated with catechol oestrogen (or clomiphene) for 10 min before addition of oestrogen. Hypothalami receiving oestrogen alone received an equal volume of 75% ethanol 10 min before addition of oestrogen. After addition of oestrogenic compounds incubations were continued for an additional 70 min and terminated by homogenisation of hypothalami in 1 ml of 5% trichloroacetic acid. Recovery of cyclic AMP was monitored by adding 0.1 pmol of ^{3}H-cyclic AMP (22 Ci mmol^{-1}). After centrifugation, 300 μl of supernatant was withdrawn, 20 μl of 1 N HCl was added, and the trichloroacetic acid supernatant was extracted four times with three volumes of diethyl ether. Residual ether was removed by heating at 56 °C for 3 min. Samples were frozen on dry ice and lyophilised. Cyclic AMP was determined by the protein binding technique of Gilman[8] as modified[9]. Protein was determined by the Miller[10] modification of the Lowry[11] technique. Inhibition (as %) of oestrogen effects by putative antagonists was calculated by the formula

$$[1-\left(\frac{(\text{oestrogen}+\text{antagonist})-\text{basal}}{(\text{oestogen})-\text{basal}}\right)] \times 100$$

Steroids, radioisotopes and reagents were from sources described before [5,6]. Values are mean ± s.e.m.

*$P < 0.02$ Student two-tailed *t* test.

†Not significantly different from no additions.

‡2-Hydroxyoestradiol added 40 min after DES.

§$P < 0.01$, Student two-tailed *t* test.

produces marked elevations in plasma luteinising hormone are also consistent with an anti-oestrogen action for the catechol oestrogens.

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Prenatal exposure of human foetuses to synthetic progestin and oestrogen affects personality

THE offspring of mothers given steroid hormones for the maintenance of at-risk pregnancy provide a human population equivalent to experimental animals for investigating early hormone effects on behaviour and personality[1]. Although patients showing the genetically induced clinical endocrine syndromes[2] associated with diminished or augmented prenatal hormone levels have been used as an alternative source of subjects, they are less useful, as results attributed to the altered hormone environment can also be attributed to genes linked to the primary source of the endocrine disfunction. Investigations using offspring of hormone-supported pregnancies are equivalent to the animal studies because in both cases the source of hormone intervention is exogenous. We report here that prenatal exposure to synthetic progestins and oestrogens affects later personality in humans.

Hormones administered to support at-risk pregnancy include both natural and synthetic progestins and oestrogens. Many of the synthetic progestins are similar chemically and in action to androgen[3,4,5] and in some cases masculinise the genitalia of exposed females. Only three samples of prenatally hormone-exposed individuals have been studied previously[3,6,7]. Unfortunately, all three of these studies suffer from design problems including lack of controls or inappropriate control groups, small sample size, and incomplete treatment information. Although individuals born of pregnancies treated with steroid hormones provide the best population with which to study the effects of the prenatal hormone environment on human behaviour, the paucity of such studies is understandable due to the difficulty of identifying and locating subjects whose mothers received hormonal intervention during pregnancy and appropriate control groups. This investigation was designed to overcome the problems of the previous studies and to expand their findings.

Six hundred records from two Los Angeles clinics and a private medical practice were reviewed. Thirty-four families were identified in which at least one pregnancy had been treated with synthetic progestin alone or in combination with oestrogen and at least one pregnancy had not been treated with these hormones. Of the 600 cases reviewed, only two children exhibited congenital abnormalities; namely a heart defect and missing fingers and toes. All available offspring of every family were evaluated for inclusion in the study. To participate, offspring of the treated and untreated pregnancies had to conform to the following criteria—the same parentage within families; at least 5 yr of age at time of study; and complete doctor and hospital records on pregnancy and delivery. Thirty-six physicians and 50 hospitals across the United States furnished the pregnancy and delivery records for the unexposed siblings and additional information on the treated pregnancies. The criteria for qualification as a treated pregnancy included a dosage minimum of 450 mg progestin (range 478–9,890); duration of at least 4 weeks (range 4–36); and treatment beginning in the first trimester of pregnancy. The final sample comprised 42 hormone-exposed subjects aged 5–17 yr (15 males and 27 females) and 42 unexposed siblings (6–18 yr; 18 males and 24 females). Birth order was balanced between exposed and unexposed subjects.

It was thought that a group of subjects could be found whose mothers were treated with a similar and consistent hormone regime during pregnancy, but review of medical records showed that treatment schedules, dosages and hormone combinations varied considerably not only among doctors but from patient to patient. Physicians frequently administered several different progestins and/or oestrogens to a single woman over the course of pregnancy. The most generally administered hormone preparations were Colprosterone, Norlutin, Delalutin, Deluteval, Provera, Provest and diethylstilbestrol. This necessitated the *post hoc* strategy of segregating subjects into two groups based on treatment regime. Group placement was defined by the ratio of progestin to oestrogen administered to the mothers of exposed subjects. The 26 members of the progestin group (P_o) were exposed to a mean ratio of 548 mg progestin to 1 mg oestrogen, when they were exposed to both hormones. Mothers of 17 subjects from this group received no oestrogen at all. Mean progestin intake per matter was 2,994 mg (range 525–9,890). The 16 subjects in the oestrogen group (O_p) were exposed to more oestrogen than progestin. The mean ratio of progestin to oestrogen was 1 : 4 and the mean total dose of oestrogen per subject was 6,119 mg (range 3,500–13,905). Mean ages for exposed subjects in the P_o and O_p groups were 12.46 and 12.06 yr respectively; mean ages of unexposed siblings for both the P_o and O_p groups were 11.8 yr at time of testing. Subjects were given the age-appropriate Wechsler intelligence scale and Cattell personality questionnaire by testers with no knowledge of the purposes of the study and the treatment category of the child. These instruments were selected for their comparability of scores across age groups.

Analysis of covariance was applied to IQ and personality scores to eliminate possible confounding influences such as heredity–family environment and prenatal and perinatal complications. The covariate of unexposed sibling score acted as a statistical control for heredity and family environment and prenatal and perinatal complications scores (severity and number of pregnancy and delivery problems) acted as controls for the at-risk nature of the treated pregnancies. Statistical application of these covariates to the scores of subjects in the exposed groups adjusted them so that they could be considered as having been generated from subjects obtained through pre-experimental random assignment to groups. This adjustment provided a *post hoc* statistical technique yielding a basis for the assumption of equality of groups before the application of treatment. Covariance analysis was also used on difference scores generated by subtracting the score of the unexposed sibling from that of the exposed subject within families—only prenatal and perinatal complications scores were applied as covariates.

Prenatal hormone exposure significantly affected subjects' responses to the personality measure. Significant differences between the P_o and O_p treatment groups were found on five personality factors: P_o group subjects were significantly more independent, sensitive, individualistic, self-assured and self-sufficient than the exposed offspring of the O_p group, while the O_p group subjects were more group-oriented and group-dependent (Table 1).

Table 1 Progestin (P_o) and oestrogen (O_p) group means for five personality factors

	Ind.	I	J	O	Q_2
P_o	7.30	6.75	7.82	2.54	6.70
O_p	5.13	5.28	4.04	5.07	3.11

Mean scores of P_o and O_p groups on five factors from the Cattell personality questionnaires (Early School Personality Questionnaire, Children's Personality Questionnaire, High School Personality Questionnaire, and 16 Personality Factors) which yielded statistically significant between group differences ($P \leqslant 0.05$). The factors are bipolar dimensions with the high-score pole versus (vs) the low-score pole for each as follows: Ind. (independent vs subdued); I (sensitive vs tough-minded); J (individualistic vs group-oriented); O (insecure vs self-assured); and Q_2 (self-sufficient vs group-dependent). The possible range in which an individual's score can fall is 1–9 with the norm by age and sex set at 5.

Similar results, consistent with the between groups analysis, were obtained when exposed subjects were compared with their unexposed siblings within families (Table 2). The exposed subjects of the P_o group were significantly more independent, individualistic, self-assured and self-sufficient than their siblings. The subjects of the O_p treatment group were significantly less individualistic, and therefore more group-oriented than their siblings and less self-sufficient and therefore more group-dependent. An integration of these findings results in the characterisation of the P_o group subjects as 'inner' or 'self' directed and those exposed to the O_p regime as 'outer' or 'other' directed in relation both to the other treatment group as well as their siblings.

The P_o group profile corresponds well to the findings of Ehrhardt & Money[3]. The mothers of their progestin exposed subjects reported high levels of self-assertive independence and self-reliance and correspondingly low frequencies of dependency and demand for succorance when describing their offspring.

In contrast to the earlier report[3], we found no significant differences on any of the scores generated from the Wechsler IQ tests. The full scale IQ for the exposed subjects of the P_o group was 121.08 with 120.12 for their siblings. The O_p group mean was 122.25 in contrast to 120.19 for their siblings. It seems that exposure to synthetic progestin alone or in combination with oestrogen has no effect on IQ, neither augmenting nor diminishing scores, when exposed subjects are compared to the base line provided by the family. The scores of both exposed and unexposed subjects, however, were 20 points higher than the norm, similar to the results obtained in the study of 10 genitally-masculinised girls[3]. The consistently elevated scores probably reflect the socioeconomic status (SES) of these families who undertook this expensive and time-consuming treatment to support at-risk pregnancy (76%, or 26 families fell into the highest SES categories, I and II, of the Hollingshead scale and no family was from the lowest category, V). As would be expected, the best predictor of the later IQ scores of exposed subjects was the IQ of their siblings (r=0.46).

Table 2 Group mean difference scores on five personality factors

	Ind.	I	J	O	Q_2
P_o	+1.19*	+0.37	+1.39*	−1.30*	+2.93*
O_p	−0.50	−0.65	−1.40*	−0.06	−3.67*

Mean difference scores of P_o and O_p groups on five factors from the Cattell personality questionnaires (see Table 1 for explanation of factors). A difference score is derived by subtracting the score of the unexposed sibling from the score of the exposed sibling. A positive score indicates that the exposed subject received a higher score than the unexposed sibling.

*Significant difference between exposed and unexposed siblings ($P \leqslant 0.05$),

Finally, Cattell has provided a configuration of six personality factors which research identified as having a significant relationship to school achievement and success[8]. The P_o group subjects exhibited high scores on three of these factors (individualistic, self-assured, and self-sufficient), whereas, the O_p groups were significantly low on two (group-dependent and group-oriented). Since there were no significant differences between the treatment groups or between exposed and unexposed siblings on the IQ tests, the groups are in effect matched for IQ. The prediction of higher school achievement in this sample of P_o regime exposed subjects is consistent with the results from the longitudinal study of prenatally progesterone exposed subjects which demonstrated significantly higher school achievement from childhood to late adolescence[6].

Our finding demonstrating a long term effect of early exposure to steroid hormones supports the investigations with animals and extends them solidly into the human realm. The data have shown that exposure to synthetic progestin and oestrogen during the foetal period significantly affects performance on a test designed to assess personality. Of course, to demonstrate conclusively a long term effect on personality in humans contingent upon prenatal exposure to various hormones, longitudinal studies of these and other individuals whose gestations were treated are necessary. The wealth of data related to early hormone influences on behaviour in animals[1] strongly suggests, however, that exposure to hormones of exogenous origin in humans should have some long term effects on such dimensions as personality and/or temperament[9].

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Synthesis and processing of ribosomal RNA by the uterus of the ovariectomised adult rat during early oestrogen action

THE rate of RNA metabolism in the uterus of the immature or ovariectomised adult rat is stimulated after the administration of oestrogen[1,2]. Although there is uncertainty concerning the nature of the RNA species synthesised by the uterus during the first hour of oestrogen action[2], it is generally agreed that by 2 h after the injection of the hormone there is a significant increase in the synthesis of ribosomal RNA[3-5]. Furthermore, Luck and Hamilton[5,6] have suggested, on the basis of sucrose gradient analysis of labelled uterine RNA, that the rate or efficiency of processing of precursor ribosomal RNA (pre-rRNA) is also increased by the hormone. We have investigated the effect of oestrogen on the metabolism of rRNA in the uterus of the ovariectomised adult rat and now report the results of gel electrophoretic analyses of uterine RNA, doubly labelled *in vivo* with (^{3}H-methyl)methionine and ^{14}C-uridine. In the atrophied uterus of the ovariectomised adult rat the rate of conversion of pre-rRNA to the definitive rRNA products was slow, and it increased with time after hormone administration reaching a maximal rate after 4 h.

Table 1 Time course for the effect of oestrogen on the ratio of RNA to DNA and the uptake and incorporation of (^{3}H-methyl)methionine and ^{14}C-uridine into RNA in the uterus of the ovariectomised rat

Hormone treatment time (h)	RNA–DNA ratio	Total tissue radioactivity (d.p.m. per mg DNA)		Radioactivity incorporated into whole-organ RNA (%)		Specific activity of isolated RNA (d.p.m. per mg)	
		^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
0	0.34	532,300	10,200	3.9	36	9,200	6,700 (1.4)*
1	0.36	535,000	15,100	4.9	32	15,800	11,700 (1.4)
2	0.39	538,900	11,000	5.2	39	18,400	10,200 (1.8)
3	0.42	672,800	11,400	5.5	40	26,300	8,600 (3.1)
4	0.45	770,700	11,900	6.3	40	44,400	9,400 (4.7)

RNA was isolated from the combined uteri of four ovariectomised adult rats that received 20 μg of 17-β-oestradiol in 0.2 ml of 1,2-propanediol (or the solvent alone), intraperitoneally (i.p.), at the times indicated before killing. All animals received 250 μCi L-(17-β-methyl)methionine (1 Ci mmol^{-1}) and 5 μCi ^{14}C-uridine (55 mCi mmol^{-1}) in 0.4 ml of water i.p. 45 min before killing. The method for isolating samples of undegraded RNA from uteri of ovariectomised adult rats was as described previously, except for the following two modifications. A second extraction of the interfacial material was performed by gently homogenising it with 4 ml of aqueous phase and stirring at 40 °C for 20 min. The phenol solutions contained an equal volume of chloroform–4% isoamylalcohol. The final yield of isolated, purified RNA averaged 67%. RNA and DNA determinations were carried out as before, as were the measurements of radioactivity incorporated[6].

*Ratio of incorporation of methionine to uridine.

Table 1 shows the effect of oestrogen, as a function of time, on the uptake and incorporation of (^{3}H-methyl)-methionine and ^{14}C-uridine into uterine RNA as well as the specific activity of the RNA isolated from uteri of ovariectomised animals that received the radioactive precursors simultaneously 45 min before death. The ratios of RNA to DNA in the various uterine homogenates revealed that oestrogen increased the concentration of RNA in the organ (compare with ref. 5). The total incorporation of (methyl-^{3}H)methionine into whole-organ RNA during the 45 min labelling period was increased 134% by hormone treatment for 4 h. Oestrogen also markedly increased the specific activity of the ^{3}H-labelled RNA isolated. It is important to emphasise that the data presented (Table 1) demonstrate that oestrogen has little or no stimulatory effect on the whole-organ uptake of methionine during the first 2 h of stimulation. After 3 and 4 h of hormone treatment the ^{3}H in the tissue was increased 26% and 45%, respectively. These observations are consistent with previous reports[5,7] and illustrate the usefulness of methionine as a precursor for labelling methylated RNAs synthesised during hormone action.

Oestrogen stimulated the uptake of ^{14}C-uridine by the uterus 48% after 1 h of treatment, 8% after 2 h, 12% after 3 h, and 17% after 4 h (Table 1). The percentage incorporation of ^{14}C-uridine into whole organ RNA was not significantly increased by hormone action. Oestrogen also increased the specific activity of the isolated ^{14}C-labelled RNA 75% after 1 h of treatment, 52% after 2 h, 28% after 3 h, and 40% after 4 h. These results are somewhat at variance with a previous report[6], in which, however, 50 μCi of ^{14}C-uridine was injected into each animal, rather than 5 μCi. The present results compare favourably with those reported by Munns and Katzman[7]. The incorporation ratio of (^{3}H-methyl)methionine to ^{14}C-uridine increased with time after hormone administration, following an initial lag period of about 1 h (Table 1). This finding is in good agreement with previous observations[3-5] that an increasing percentage of the newly synthesised RNA formed by the uterus during the first few hours following oestrogen stimulation is methylated.

The absorbance and radioactivity profiles of the gel electrophoretic analyses of the samples of labelled RNA isolated in the experiment described in Table 1 are shown in Fig. 1. The absorbance profiles of all the RNA samples analysed were essentially the same. The ^{14}C profiles (not shown) revealed that uridine had been incorporated into RNA very heterogeneous in size in both the control and hormone-stimulated uterus. No differences in the shapes of the ^{14}C profiles could be attributed to oestrogen. The ^{3}H profiles, however, reflected differences in the relative amounts of the various classes of labelled precursor, intermediate and mature rRNA species present in the uterus at various times after the injection of the hormone. Incorporation of (^{3}H-methyl)methionine into 45S, 32S, 28S, 21S and 18S rRNAs took place during the 45-min labelling period in both control and hormone-treated animals. In the unstimulated uterus the percentage of ^{3}H found in the precursor 45S and 32S rRNAs totalled 49% of the label incorporated into all rRNA species. But, as the time of the

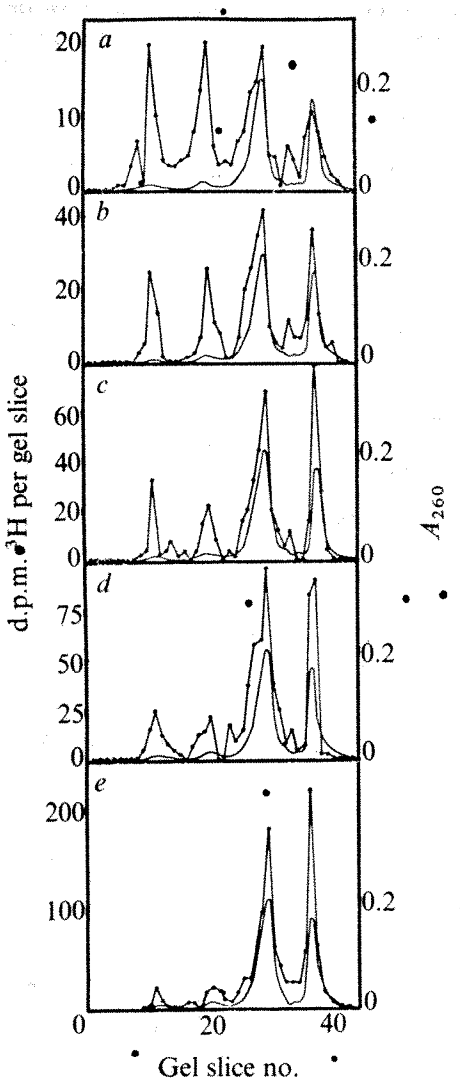

Fig. 1 Absorbance and radioactivity profiles of isolated, uterine RNA, labelled *in vivo* with (³H-methyl)methionine, as a function of time after administration of oestrogen. Experimental details are as in Table 1. Rats received *a*, no hormone, or oestrogen for *b*, 1 h; *c*, 2 h; *d*, 3 h; or *e*, 4 h. (●—●), ³H; ——A_{260}. Samples of doubly-labelled RNA, of exactly 0.5 A_{260}, were fractionated as described by Caston and Jones[8] on 1.8% polyacrylamide–1% agarose gels prepared in quartz tubes (90×5 mm inside diameter). Electrophoresis was for 2½ h at 5 mA per gel. Unlabelled 28S and 18S rRNAs were isolated from rat liver ribosomes and initially used as markers, as was 45S pre-rRNA isolated from nuclei of Novikoff ascites cells. Gels were scanned at 260 nm in a Gilford spectrophotometer and sliced into 2-mm slices. Each slice was placed in a counting vial and 0.1 ml of 30% ammonium hydroxide added to hydrolyse the RNA. After standing overnight at room temperature 1 ml of NCS tissue solubiliser (Amersham/Searle) was added to each vial and all vials were shaken for 1 h. Scintillation fluid, 10 ml, (5 g PPO and 0.1 g POPOP per litre of toluene) was added to each vial and the radioactivity determined to an error of 5% or less in a Beckman liquid scintillation spectrometer. A ³H counting efficiency of 44% was obtained.

hormone treatment increased this percentage decreased until 4 h after oestrogen administration only 12% of the total amount of methyl-labelled rRNA formed was present in the 45S and 32S fractions (Table 2). Longer periods of stimulation by the hormone did not significantly alter this distribution of the label among the 45S, 32S, 28S and 18S fractions. Labelled precursor was available to the uterine cells during the entire 45-min labelling period as evidenced by the presence of (³H-methyl)methionine in the organ at the time of killing of the animals. These data demonstrate that during the first 4 h of oestrogen action in the uterus of the ovariectomised adult rat the amount of new methylated 28S and 18S rRNA formed from the methylated 45S precursor per unit time is increased nearly fourfold.

We have routinely observed a methyl-labelled fraction slightly larger than 45S in uterine RNA samples isolated from the control animals which was not present following oestrogen stimulation (see Fig. 1*a*). Interestingly, Galibert *et al.*[9] have reported that there are three distinct components in the 45S pre-rRNA fraction of L-cells separable by electrophoresis through 1.7% polyacrylamide gels. Whether this heterogeneity represents a population of slightly different initial precursor molecules or early processing intermediates remains to be elucidated.

Table 2 Effect of oestrogen on the incorporation of (³H-methyl)-methionine into ribosomal RNAs by the rat uterus

Hormone treatment time (h)	Ribosomal RNA fraction 45S (%)	32S (%)	28S (%)	18S (%)
0	21	28	32	19
1	19	18	39	24
2	12	11	47	30
3	10	9	51	30
4	5	7	55	33

The percentages in this table are calculated from the radioactivity profiles shown in Fig. 1. The label in the 45S fraction was computed by summing the d.p.m. in gel slices 5–15; the 32S fraction by summing gel slices 16–22; the 28S fraction, gel slices 23–32; and the 18S fraction, gel slices 33–41.

Post-transcriptional mechanisms for regulating rRNA synthesis have recently been proposed in phytohaemagglutinin-stimulated lymphocytes[10,11], during compensatory renal hypertrophy[12], in a mutant of BHK cells[13,14] and in liver[15]. It is not yet clear, however, whether the accelerated rate of processing of pre-rRNA to 28S and 18S rRNAs in the oestrogen-stimulated uterus is due to more efficient methylation and cleavage, a more efficient processing pathway, or is determined by increased rates of rRNA synthesis[16].

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Thioredoxin and glutathione regulate photosynthesis in chloroplasts

Two recently found chloroplast proteins act in the ferredoxin-linked regulation of enzymes of photosynthetic carbon dioxide assimilation[1,2]. We report here the identification of one of these proteins, earlier termed assimilation regulatory protein b (ARP$_b$), as thioredoxin—a protein functional in the synthesis of DNA and previously not known in chloroplasts [3–5]. The other protein, assimilation regulatory protein a (ARP$_a$), is a new enzyme that catalyses the reduction of thioredoxin by reduced ferredoxin. We have renamed this protein 'ferredoxin–thioredoxin reductase.' In addition to identifying these proteins found earlier, we present evidence for a regulatory function of glutathione in chloroplasts. These findings permit for the first time the formulation of a light-dependent mechanism for protein-mediated enzyme regulation. In this mechanism, key regulatory enzymes are activated (reduced) photochemically via the ferredoxin–thioredoxin system and are deactivated (oxidised) in the dark via oxidised glutathione. The evidence indicates that photoreduced ferredoxin, in the presence of ferredoxin–thioredoxin reductase, controls the level of reduced thioredoxin required for enzyme activation. Glutathione reductase and glutathione peroxidase seem to control the level of oxidised glutathione available for enzyme deactivation.

Fructose 1,6-bisphosphatase of chloroplasts was assayed by measuring the release of inorganic phosphate (P$_i$) from the carbon-1 of fructose 1,6-bisphosphate[1,6]. Thioredoxin from *Escherichia coli* could fully replace native ARP$_b$ in the light-induced activation of chloroplast fructose 1,6-*bis*-phosphatase (Table 1). In this system, enzyme activation required ferredoxin, reduced photochemically with chloroplast fragments, and ARP$_a$ in addition to either ARP$_b$ or *E. coli* thioredoxin. ARP$_b$ and *E. coli* thioredoxin were also found to be equally effective when their capacity for phosphatase activation was tested in the presence of the non-physiological sulphydryl reagent dithiothreitol and in the absence of reduced ferredoxin and ARP$_a$ (refs 1, 6; Table 2).

The dithiothreitol assay was used previously to demonstrate that ARP$_b$ exists in the major types of living cells[2]. ARP$_b$ was found not only in photosynthetic plant and bacterial cells but also in their non-photosynthetic counterparts and in animal cells. In view of the ability of *E. coli* thioredoxin to replace ARP$_b$ in activation of the chloroplast fructose bisphosphatase, it seems likely that the active component found earlier in the different types of cells was thioredoxin. The known wide distribution of thioredoxin is in accord with that conclusion[7].

Table 1 Interchangeability of chloroplast ARP$_b$ and *E. coli* thioredoxin in the activation of chloroplast fructose 1,6-*bis*-phosphatase by photoreduced ferredoxin

Treatment	nmol P$_i$ released per min Chloroplast ARP$_b$	 *E. coli* thioredoxin
Light, complete	12	10
Light, minus ARP$_b$ or thioredoxin	2	2
Light, minus ferredoxin	4	2
Light, minus ARP$_a$	1	1
Light, minus fructose 1,6-bisphosphatase	0	1
Dark, complete	2	1

The reaction was carried out at 20 °C in Warburg vessels containing (in the side arm) 6 μmol of sodium fructose 1,6-*bis*-phosphate and (in the main compartment) 17 μg of fructose 1,6-*bis*-phosphatase dimer[26], spinach chloroplast fragments (P$_1$S$_1$) equivalent to 0.1 mg chlorophyll[10], 0.12 mg *Spirulina maxima* ferredoxin[27], 70 μg ARP$_a$ (in an aliquot containing 3 μmol 2-mercaptoethanol), and the following (μmol): Tris-HCl buffer (*p*H 7.9), 100; neutralised reduced glutathione, 5; sodium ascorbate, 10; 2,6-dichlorophenolindophenol, 0.1; MgSO$_4$, 1.0. ARP$_b$, 25 μg, or *E. coli* thioredoxin, 20 μg, were added as indicated. Final volume, 1.5 ml. After equilibration with nitrogen for 6 min, vessels were incubated for 5 min in the light (20,000 lx) or in the dark. The reaction was started by adding fructose 1,6-*bis*-phosphate from the side arm and was continued for 30 min under illumination or in the dark. The reaction was stopped by adding 0.5 ml of 10% trichloroacetic acid. After centrifuging off the precipitate, the P$_i$ released was estimated by a modified Fiske–SubbaRow procedure[10]. ARP$_a$ and ARP$_b$ were purified as before[1,6]. ARP$_a$ was then further purified by DEAE–cellulose and hydroxyapatitecol umn chromatography (details to be published elsewhere).

Table 2 Equivalence of chloroplast ARP$_b$ and *E. coli* thioredoxin in the activation of chloroplast fructose 1,6-*bis*-phosphatase by dithiothreitol

Treatment	nmol P$_i$ released per min
Chloroplast ARP$_b$, 12 μg	50
E. coli thioredoxin, 10 μg	47
Minus ARP$_b$ or thioredoxin	7

The reaction was carried out in air at 25 °C in test tubes containing, in 0.5 ml, fructose 1,6-*bis*-phosphatase, 9 μg; ARP$_b$ or thioredoxin as indicated, and the following (μmol): Tris–HCl buffer (*p*H 7.9), 50; MgCl$_2$, 0.5; dithiothreitol, 2.5; sodium fructose1,6-*bis*-phosphate, 3. The reaction was started by adding fructose 1,6-*bis*-phosphate and was continued for 30 min. The reaction was stopped by adding 2 ml of a mixture of the Fiske–SubbaRow reagents used to analyse P$_i$ (see Table 1).

The observation that *E. coli* thioredoxin is active in assays specific for ARP$_b$ suggests that ARP$_b$ might substitute for thioredoxin in the synthesis of DNA. This was confirmed by the finding that chloroplast ARP$_b$ showed thioredoxin activity in the *E. coli* ribonucleotide reductase assay system. Chloroplast ARP$_b$ replaced native thioredoxin to a low but significant extent in the enzyme-mediated reduction of ribonucleotides to their deoxy derivative. The low activity of chloroplast ARP$_b$ in this assay is attributed to its weak affinity for the enzymes of DNA synthesis in *E. coli*.

Our results show unforeseen similarities between two proteins that were discovered in very different lines of research—thioredoxin was found in studies on the synthesis of DNA in *E. coli* and ARP$_b$ was found in studies on the light-dependent regulation of enzymes in chloroplasts. It now seems that these two proteins are functionally similar. We therefore consider a revision of our original nomenclature timely and designate ARP$_b$ 'chloroplast thioredoxin'. By analogy, the protein earlier called ARP$_a$ is, on the basis of its ability to catalyse a thioredoxin-dependent oxidation of reduced ferredoxin (Table 3), designated 'ferredoxin–thioredoxin reductase'. We found no indication that chloroplasts contain an *E. coli*-type NADP-thioredoxin reductase[8] that links NADPH to the activation of fructose 1,6-*bis*-phosphatase.

The ferredoxin–thioredoxin system constitutes a new mechanism which, in chloroplasts, links light to the regulation of certain enzymes of carbon dioxide assimilation. An important aspect of this or any other system of enzyme regulation is the

Table 3 Identification of chloroplast ARP$_a$ as ferredoxin–thioredoxin reductase

Component added to oxidise photoreduced ferredoxin	nmol ferredoxin oxidised per min
None	0.0
ARP$_a$	0.6
Chloroplast thioredoxin	0.9
ARP$_a$+chloroplast thioredoxin	12.0

The reaction mixture contained, in 1.2 ml, *Spirulina maxima* ferredoxin, 0.18 mg; chloroplast fragments[10] (P$_1$S$_2$) equivalent to 5 μg chlorophyll; and the following (μmol): Tris-HCl buffer (*p*H 7.9), 100; MgSO$_4$, 1; sodium ascorbate, 10; and 2,6-dichlorophenolindol phenol (DPIP), 0.1. ARP$_a$, 230 μg (in an aliquot containing 6 μmol 2-mercaptoethanol) was added as indicated. Samples were gassed with argon for 5 min and transferred anaerobically to a glass cuvette of 2-mm light path. After fully reducing the ferredoxin photochemically by illumination with red light (Corning 2-58 filter), the light was turned off, and the rate of the oxidation of ferredoxin was followed in the dark. The extent of oxidation of ferredoxin was measured by increase in absorbance at 420 nm with a modified Beckman Model DU monochromator[29].

Table 4 Deactivation of chloroplast fructose 1,6-bisphosphatase by oxidised glutathione

Reductant used for activation	% of control activity after adding glutathione
Reduced ferredoxin*	53
Dithiothreitol†	5

*The reaction mixture was as in Table 1 except that 25 μmol oxidised glutathione were added to the second side arm and 104 μg ferredoxin–thioredoxin reductase (ARP_a) (plus 3 μmol 2-mercaptoethanol) were used. Vessels were equilibrated with nitrogen for 6 min and incubated 10 min in the light (20,000 lx). The reaction was started by adding fructose 1,6-*bis*-phosphate from the first side arm. After 10 min under illumination, the lights were extinguished, oxidised glutathione (or water) was added from the second side arm, and the reaction was continued for 20 min in the dark. The reaction was stopped and P_i was analysed as in Table 1. 15 nmol of P_i were released per min in the control treatment.

†Fructose 1,6-*bis*-phosphatase was activated (and deactivated) before assay. Fructose 1,6-bisphosphatase, 17 μg, was incubated in 0.1 ml of a mixture containing 0.1 M Tris-HCl buffer (*p*H 7.9), 10 mM dithiothreitol, and 25 μg chloroplast thioredoxin (ARP_b). After 10 min, 2.5 μmol of oxidised glutathione (or water) was added. After a further 20 min, the enzyme was then injected into 0.9 ml of the assay mixture containing 1.3 units of glucose 6-phosphate dehydrogenase, 4.2 units of glucose-6-phosphate isomerase, and the following (μmol): Tris-HCl buffer (*p*H 7.9), 40; $MgSO_4$, 1; fructose 1,6-*bis*-phosphate, 6; and NADP, 1.5. Fructose 1,6-*bis*-phosphatase activity was followed by measuring the reduction of NADP (increase in absorbance at 340 nm) with a Cary model 14 spectrophotometer. Temperature, 22 °C. 29 nmol of P_i were released per min in the control treatment.

way in which the component enzymes are converted from an active to a less active (deactivated) state. Until now, no deactivation mechanism was known for the ferredoxin–thioredoxin system. As seen in Table 4, glutathione—a sulphydryl–disulphide component of most if not all living cells[9]—functioned in that capacity. The oxidised form of glutathione deactivated the fructose *bis*-phosphatase that had been activated previously by thioredoxin reduced either photochemically with the ferredoxin system or non-enzymically with dithiothreitol. Under the same conditions, reduced glutathione had no effect.

The effectiveness of oxidised glutathione in deactivation suggests that *in vivo* a defined ratio of oxidised to reduced glutathione gauges the activity of enzymes, such as fructose bisphosphatase, in response to changing conditions in the chloroplast. This ratio, in turn, may be determined by two enzymes present in the soluble fraction of chloroplasts[10], glutathione reductase [11] (equation 1) and glutathione peroxidase[12] (equation 2).

$$NADPH_2 + \text{glutathione}_{\text{oxidised}} \xrightarrow[\text{reductase}]{\text{glutathione}} 2\ \text{glutathione}_{\text{reduced}} + NADP \qquad (1)$$

$$2\ \text{glutathione}_{\text{reduced}} + H_2O_2 \xrightarrow[\text{peroxidase}]{\text{glutathione}} \text{glutathione}_{\text{oxidised}} + 2\ H_2O \qquad (2)$$

The specific activity in μmol substrate converted per mg chlorophyll per h was 9 for glutathione reductase[13] and 12 for glutathione peroxidase[14]. Because of its relatively high activity, it seems possible that glutathione peroxidase could, in addition to its regulatory role described here, function together with glutathione reductase in removal of hydrogen peroxide produced in chloroplasts. Accordingly, glutathione peroxidase would replace catalase, which seems to exist mainly outside rather than inside chloroplasts[15,16]. Our finding of 1 mM glutathione[17] in chloroplasts is consistent with such a function for glutathione peroxidase.

Although the physiological agent for enzyme deactivation seems to be oxidised glutathione, other oxidants were also effective in this reaction. Sodium tetrathionate ($Na_2S_4O_6$) and the oxidised form of ascorbic acid (dehydroascorbic acid) showed deactivation effects similar to those reported in Table 4 for oxidised glutathione. In view of the high concentration of ascorbic acid in chloroplasts[18,19], these results indicate that dehydroascorbic acid, produced from ascorbic acid through oxidation, could possibly be involved in the control of enzyme activity.

Considering the wide distribution of ferredoxin and thioredoxin, a comment on the possible application of the ferredoxin–thioredoxin system to other organisms seems appropriate. It is possible that a mechanism of the type described could be used for the regulation of cellular processes in other organisms. Furthermore, because many cells are enzymically fitted to reduce thioredoxin independently of ferredoxin, by way of NADP, the presence of ferredoxin is not prerequisite to the operation of such a system. For example, a mechanism of the NADP-thioredoxin type could account for the thioredoxin-mediated reduction of proteins (for which the reported reduction of insulin provides a model[20]) or for modification of proteins through the formation of a stable thioredoxin–protein complex (as found for a specific DNA polymerase in phage-infected *E. coli* cells[21]).

The present results provide evidence for a new mechanism whereby specific enzymes are activated by reduction (via thioredoxin) and deactivated by oxidation (via oxidised glutathione). In chloroplasts this 'oxidation–reduction' mechanism acts in conjunction with the ion-mediated and effector-mediated mechanisms discussed previously[1,22] which link enzyme regulation to the absorption and utilisation of light. In the light, electrons from chlorophyll are transferred to ferredoxin and then, via a specific reductase enzyme, to thioredoxin (Fig. 1). Reduced thioredoxin, in turn, reduces and thereby activates certain regulatory enzymes of chloroplasts (fructose 1,6-*bis*-phosphatase, sedoheptulose 1,7-*bis*-phosphatase[23], and perhaps NADP-glyceraldehyde 3-phosphate dehydrogenase and phosphoribulokinase[1,24]). Our evidence indicates that, at least in the case of NADP-glyceraldehyde 3-phosphate dehydrogenase, reduction (activation) is accompanied by the formation of a stable thioredoxin–enzyme complex.

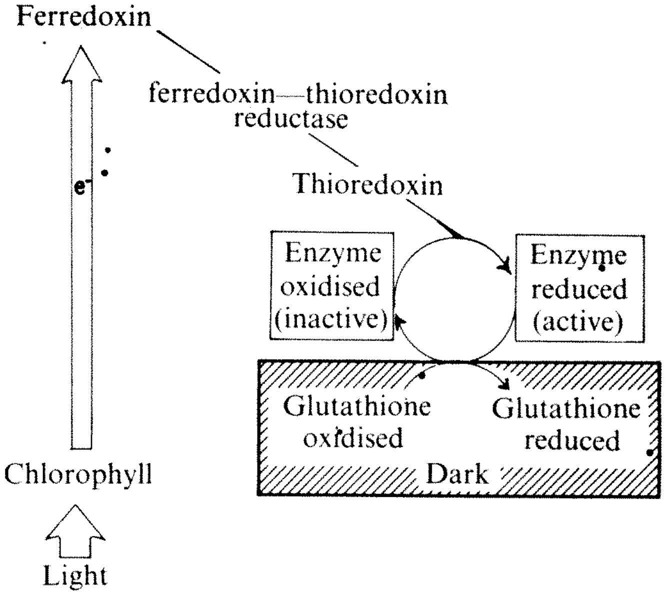

Fig. 1 A mechanism for the light-dependent regulation of enzymes in chloroplasts. The component[30–32] before the indicated soluble ferredoxin that accepts electrons from photoactivated chlorophyll is not shown.

In the dark, the activated (reduced) enzymes are deactivated (oxidised) by an oxidant such as oxidised glutathione. Figure 2 shows that, in the presence of hydrogen peroxide, glutathione peroxidase could act in the dark to convert reduced glutathione to the oxidised form that is functional in deactivation. It is likely that limited enzyme deactivation would occur also in the light in response to transient changes in the chloroplasts.

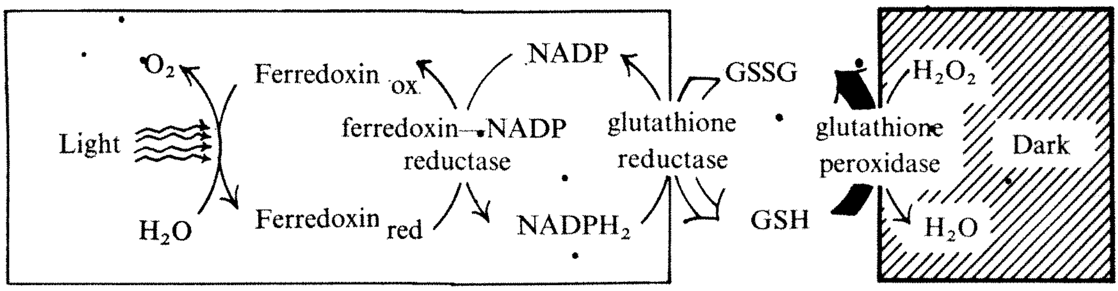

Fig. 2 A mechanism for controlling the ratio of oxidised (GSSG) to reduced (GSH) glutathione in chloroplasts.

The high concentration of reduced NADP prevailing in those conditions[25], however, would tend to impede such deactivation by converting the oxidised glutathione to its reduced form with the chloroplast enzyme glutathione reductase.

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Was photoresolution of amino acids the origin of optical activity in life?

SINCE Pasteur showed that the amino acid asparagine in plants was purely one optical isomer, constant interest has centred around the question of optical activity in life. The quantities of D-amino acids in the biosphere are practically negligible and none has ever been found in any enzyme or other protein, whereas the abundance of L-amino acids is well known. Many of the molecules important to living processes can be synthesised[1] in conditions which may have prevailed on a primitive planet and it is easier to suggest a prebiotic synthesis of a racemate than to explain an enantiomeric selection. Whereas many workers claim the clue might be found in various stereoselective mechanisms, likely to have operated already at the stage of the 'chemical evolution[2-5]', no example of the selection of a biologically crucial compound by means of circularly polarised light has been previously reported. Apart from an effect due to an enantiomorphous relationship between matter and antimatter[6-8], this is the only mechanism with selection capacity in a large system (the Earth)[9]. We report here that tartaric acid, α-alanine and glutamic acid partially resolve under circularly polarised irradiation. The L-amino acid dominance in the biosphere can by analogy, be explained by a selection caused by circularly polarised interstellar ultraviolet radiation.

Pairs of identical racemic aqueous solutions were simultaneously subjected to left and right circularly polarised light (quartz collimators/Glan prisms/Fresnel rhombs) from a Xe (450 W quartz, Osram) lamp, λ > 200 nm (aqueous infrared filter) and compared afterwards with respect to circular dichroism difference at the wavelengths of photolysis (Jasco J-41 dichrometer). Results are given in Table 1 and Fig. 1.

Fig. 1 Typical circular dichroism spectra (× 10^6) recorded on *a*, racemic tartric acid and *b*, glutamic acid after irradiation with right and left circularly polarised light. *c*, Recorded ΔCD deflections (× 10^6) at 210 nm in glutamic acid at different irradiation times (different symbols for different experiments).

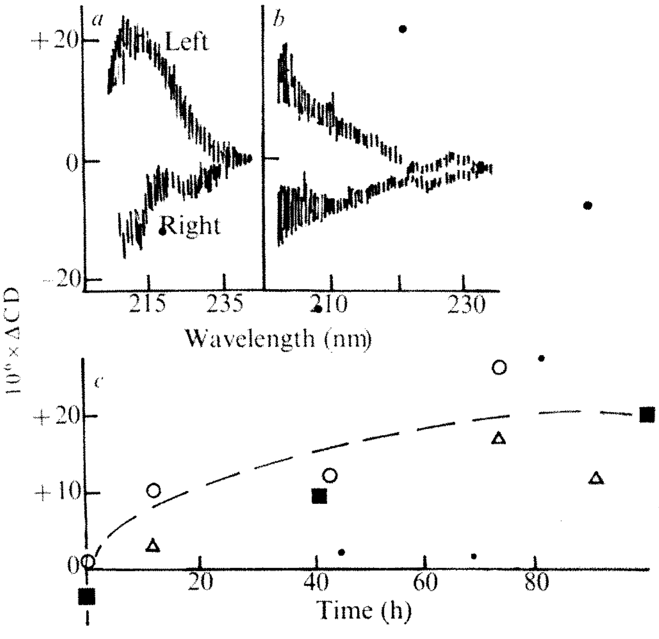

Table 1 Observed and calculated selection

Specimen	Wavelength (nm)	$\lvert\Delta\varepsilon/\varepsilon\rvert$ of pure enantiomer	Irradiation time (h)	Conversion (inversion plus decomposition) (%)	$10^6\times\Delta$CD normalised to $A_0 = 1$, between left and right circularly irradiated solutions (absorbance units)	Enrichment yield in each sample (%)	
DL-tartaric acid,	250	0.002		> 20		0.11*	(0.8)†
1 cm, 5×10^{-3} M	230	0.020					
in H_2O	220	0.020	30		42 ± 5		
	215($\Delta\varepsilon_{max}$)	0.020					
DL-alanine,	210–230	(0.010)	72		0	0	(0.32)†
1 cm, 10×10^{-3} M	203($\Delta\varepsilon_{max}$)	0.007					
in H_2O	210–230		160	> 20	9 ± 5	0.06*	(0.32)†
DL-glutamic	230	(0.011)		20–40		0.11*	(0.35)†
acid, 1 cm,	225	0.008					
10×10^{-3} M in	210	0.008	100		18 ± 5		
H_2O	202($\Delta\varepsilon_{max}$)	0.007					
	210	0.008	504	52	36 ± 5	0.22*	(0.35)†

*Defined as $100\times(\mathrm{CD_{obs}}/\mathrm{CD_{pure\ enantiomer}}) \approx 50\ \Delta\mathrm{CD}/(\Delta\varepsilon/\varepsilon)$.

†Theoretically possible figures in parentheses, obtained as $100\times(\mathrm{CD_{theor}}/\Delta\varepsilon\ Cl) \approx 40\ (\Delta\varepsilon/\varepsilon)^2A/Cl = 40\ \Delta\varepsilon/\varepsilon$.

Due to uncertain lamp intensity in the active range (200–230 nm) relevant quantum yields could not be estimated.

It is realistic to assume that the activation is achieved either by photodecomposition or by photoinversion leading to excess of the enantiomer that has positive circular dichroism at the photolytical wavelength, when right circularly polarised light is used and vice versa. Some amino acids may undergo photo-deamination to yield cinnamic acids, where the unsaturated character can cause obscuring absorption preventing complete photolytic yield. When optically active tartaric acid and aliphatic amino acids were subjected to non-polarised ultra-violet light ($\lambda > 200$ nm) absorptive products were generally not observed and the rate of loss of optical activity indicated a simultaneous photoracemisation. In the case of pure photo-decomposition the maximum absolute activity is expected when 63% of the material is decomposed, by a circular dichroism deflection[10]

$$|\mathrm{CD}| = 0.37\times(\Delta\varepsilon/\varepsilon)^2A_0 \qquad (1)$$

($\Delta\varepsilon = \varepsilon_l-\varepsilon_r$ and ε are molar, M^{-1} cm^{-1} circular dichroism and absorption coefficients, A_0 the initial absorbance. As was shown elegantly by Kagan[11] 100% purity can, however, be obtained at the end of the reaction. For photoracemisation a steady state

$$|\mathrm{CD}| = 0.5(\Delta\varepsilon/\varepsilon)^2A_0 \qquad (2)$$

should be reached. In both cases it is presumed that only the excited molecule undergoes reaction (and that it does so due to the dichroic transition), that is, that no radical attacks or sensitising effects occur. By the quadratic appearance of the dissymmetry factor $\Delta\varepsilon/\varepsilon$ the possibilities of experimental detection are restricted to molecules with bands having $|\Delta\varepsilon/\varepsilon| > 3\times10^{-3}$ due to the resolution of present day instruments, which is $\pm3\times10^{-6}$ absorbance units at unit absorbance. That the observed optical activity is due to optical selection among the starting materials and not to any optically active secondary product is indicated by the complete extinction of optical activity when the pure isomers are irradiated. The final proof was obtained by the observation of a constant line-shape of the CD-spectrum during the decay period.

Finally, it should be pointed out that a spectral requirement $\lambda > 200\pm30$ nm on the incident radiation is in excellent agreement with the current opinion of the composite ultraviolet absorption of the primitive atmosphere (due mainly to water vapour and carbon dioxide)[12]. The L-amino acids (except the cyclic imino-acid proline) are thus selected by this type of light (when right circularly polarised), while for instance nucleic acids should require $\lambda > 260$ nm for an effective yield (according to the circular dichroism spectrum with wings of both signs). We are also studying amino acid complexes with evolutionarily interesting metals and where magnetic dipole allowed d–d transitions (for example Cr(III)) can be expected to strongly enhance the photoresolution efficiency.

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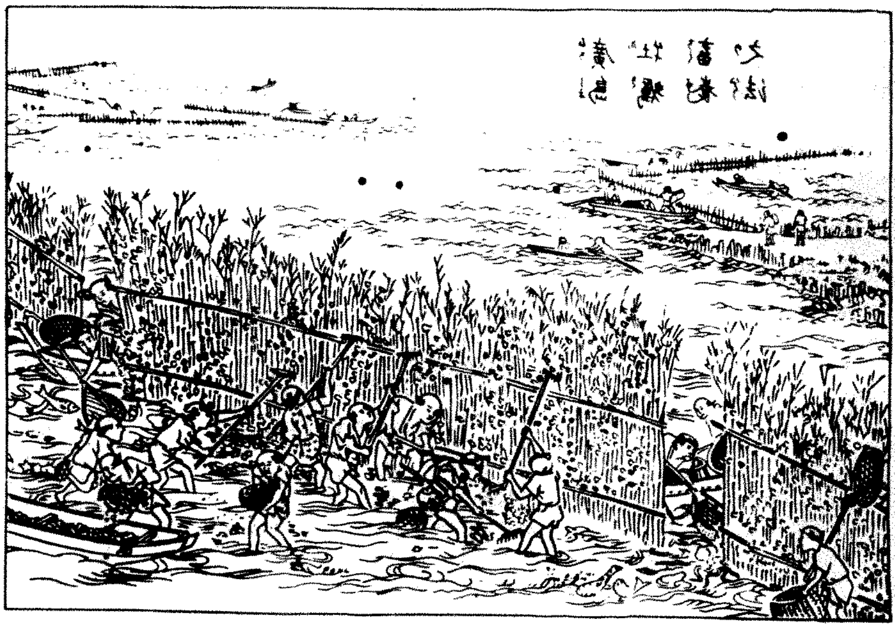
reviews

Aquaculture methods

C. M. Yonge

Farming Marine Organisms Low in the Food Chain. Farming Cupped Oysters of the Genus Crassostrea. *Farming the Flat Oyster of the Genus* Ostrea. *Farming Marine Fishes and Shrimps.* (Developments in Aquaculture and Fisheries Science, Vols 1–4.) By P. Korringa. (Elsevier: Amsterdam, Oxford and New York, 1976.) $30.95; Dfl.80 each volume.



Ancient method of oyster culture in Hiroshima Prefecture

AT this time of increasing competition for already over-exploited stocks of fish, any increase in marine productivity by aquaculture demands attention. No one is better qualified to write about this than Professor P. Korringa, Director, Netherlands Institute for Fishery Investigations. Responsible for restoration of the Dutch oyster beds following shell disease in the 1930s, he must since have visited every significant area of marine cultivation. This reviewer has memories of visits in his company to areas of oyster and mussel cultivation in France, Spain and Italy.

His descriptions of culture methods are based on actual procedures within selected aquacultural enterprises. For each he supplies a biological, geographical, oceanographic and even legal background—thus sheltered, pollution-free waters and security of tenure are all involved. Farming procedures include control of predators, parasites and disease. Economic problems including marketing are finally assessed.

Description of culture methods begins with that of the red seaweed 'Nori' (*Porphyra*) in Japan and concludes with that of rainbow trout and salmon in Norway. Cultivation of bivalve molluscs is inevitably the major theme; mussels (*Mytilus* spp.) clams and, above all, species of the flat oyster, *Ostrea*, are covered together with those of the ecologically more tolerant cupped oyster, *Crassostera*, which are everywhere replacing them.

Mussels, strangely neglected in North America and Japan, are reared on stakes in the long established 'bouchot' system in France, by mass transplantation in the Netherlands, and with impressive productivity in the sheltered "rias" in north-west Spain, where settlement and growth are on ropes suspended from moored pontoons or catamarans.

The great French oyster industry was initially based on studies of the surviving remnants of Roman culture methods in the Mediterranean. Large scale cultivation, between tide marks—first of *Ostea edulis*, then of the Portuguese *Crassostrea angulata* and now of the ubiquitous Japanese, *C. gigas*—was established; and its methods have now been widely copied throughout the world. The highly successful suspended culture method was initiated in Japan same fifty years ago, at first to offset restrictions caused by limited intertidal space.

All these methods involve collection of naturally produced spat in areas of relatively still water and subsequent growth in water rich in phytoplankton. Only in hatcheries (about which Dr Korringa has surprisingly little to say), where oysters are induced to spawn and the larvae and spat fed on reared algal species, can full culture be established.

With fish, only the cultivation of herbivores—here described for mullets (*Mugil*) in Israel and in 'valli' round the mouth of the Po in the Adriatic, and for milk fish (*Chanos*) long cultivated in Indonesia—represents economic production of animal protein. Japanese cultivation of the carnivorous yellow-tail (*Seriola quinqueradiata*) and red sea bream (*Chrysophrys major*)—with that of the no less carnivorous penaeid prawns (*Penaeus japonicus*)—involves conversion, with much accompanying labour, of lower into higher or more acceptable forms of animal protein. The result is an expensive luxury product.

Conditions are somewhat different with eel culture in Japan—supplying such a demand that elvers are imported from so far afield as Europe—and salmonid rearing in Norway and Scotland. These have the advantage of supplying an insatiable demand at a lower cost than that involved in collection of these fish in Nature.

These admirably produced and illustrated books are a pleasure to review. They will be of the greatest value to aquaculturists; nothing is described that has not been personally inspected and judged in the light of Dr Korringa's uniquely wide experience. □

Sir Maurice Yonge, formerly Professor of Zoology at Bristol and then Glasgow, is now Honorary Fellow in Zoology at the University of Edinburgh, UK.

Molecular genetics handbook

Handbook of Genetics. Vol. 5: Molecular Genetics. Edited by R. C. King. Pp.xi+667. (Plenum: New York and London, 1976.) $51.

THE fifth volume of the *Handbook of Genetics* in many ways belies its title. The book is in four sections devoted to the molecular organisation of chromosomes (six chapters), gene transcripts (five chapters), chloroplasts and mitochondria (five chapters), and a single chapter in a section called mutant enzymes. Only the chapters in the section on chloroplasts and mitochondria seem truly to combine both a molecular and genetical approach and deserve the title molecular genetics.

Particularly disappointing is the *pot pourri* section on molecular organisation of chromsomes, which manages to present a somewhat incoherent treatment of the subject in spite of excellent reviews of chromosomal protein structure and function, and of organisation and size of replicons. Individual chapters on repeated DNA in eukaryotes, cytological localisation of repeated DNAs, chromosomal proteins, organisation of genetic material in the macronucleus of hypotrichous ciliates, histones of sperm, and organisation and size of replicons, do not easily relate to each other and the section as a whole presents no overall picture of chromosome organisation. The chapter on repeated DNA in eukaryotes, by limiting itself to molecular renaturation studies, manages to ignore much interesting and relevant data provided by sequencing, restriction site mapping and electron microscopy of repeated DNAs.

The chapter on chromosomal proteins, although admirable in many ways, contains no reference to the unit of chromatin (nucleosome) and models which have been proposed for its structure. Such an omission can hardly be of the authors own making since it seems evident from the reference list that this chapter was complete by the end of 1973 (the only 1974 paper quoted is by the authors themselves) and the same seems to hold for the majority of chapters in this section; one exception being the chapter on macronuclear DNA of hypotrichs, which has references to 1976. Judging by the latest references in chapters of other sections they were completed towards the end of 1974 or beginning of 1975, although the chapter on mutant enzymes has no references after 1972. It is a pity that the relatively long lag between writing and publishing the chapters in section one has reduced its effectiveness as a contemporary review of a rapidly changing area of investigation.

The other sections are more successful. Section two, gene transcripts, has chapters on nuclear RNA, RNA transcription and ribosomal protein assembly in *Drosophila melanogaster,* cytoplasmic mRNA, tRNAs, and eukaryotic ribosomes; whereas section three, chloroplasts and mitochondria, has chapters on chloroplast DNA (physical and genetic studies), chloroplast ribosomes, nucleocytoplasmic interaction in *Acetabularia,* mitochondrial DNA, and mitochondrial ribosomes. The chapters on cytological localisation of repeated DNAs, histones of sperm, transfer RNAs, and mutant enzymes are little more than exhaustive catalogues of the structure, location or primary sequence of particular macromolecules.

In spite of some omissions which might have been included in a book with this title—for instance, considerable space is devoted to histones but no mention is made of histone genes, and similarly there is no mention of tRNA genes although all the known tRNA sequences are tabulated—there is a great deal of worthwhile and well presented material. As a reference book in certain areas, it should prove valuable.

The book has an author and subject index. But the subject index has innumerable deficiencies, and I pick one example to illustrate this point. The only reference to "globin" contains an interesting cross-reference "see Immunoglobin". There is, however, no entry under "Immunoglobin"; but under "Immunoglobulin" there is a reference to "chain of rabbit reticulocyte"! **Peter Ford**

Peter Ford is Lecturer in the Department of Molecular Biology, University of Edinburgh, UK.

Chemical relaxation

Relaxation Kinetics. By C. F. Bernasconi. Pp. xi+288. (Academic: New York and London, 1976.) $29.50; £20.95.

THERE can be no getting away from it —a large proportion of chemical reactions are fast. Generations of students may have obtained their grounding in chemical kinetics with reactions whose half-lives lie conveniently in the time range of minutes or hours, but the fact is that such reactions are the exception rather than the rule. One has only to recall the particular premium placed on the time factor in many biologically and industrially important reactions to appreciate the significance of our being able to measure the kinetics of rapid reactions.

It is not possible to apply classical kinetic techniques to many of these reactions but over the past fifteen years or so a variety of methods have been developed which have made most of them susceptible to study. All of these methods involve electronic equipment and the prospect of having to build one's own apparatus, together with the fact that a rather different approach is generally required in the analysis of the data, has tended to scare away all but the most physically-minded chemists. Times are, however, changing and it is thanks to the increasing availability of suitable commercial equipment and monographs such as Dr Bernasconi's that more and more chemists are reporting rate constants and activation parameters where in the past they would have used some such phrase as "too fast to measure".

The first two-thirds of Dr Bernasconi's book deals with the general theory of chemical relaxation and is largely given over to the problem of relating the relaxation time (or times) and the rate constants for different mechanistic schemes. Also included are a couple of chapters (out of ten) on the topic of relaxation amplitudes. The remainder of the book is concerned with the various experimental methods.

The author says that he has attempted to write a comprehensive treatment of the subject in the language of the organic or physical–organic chemist and in this I think he has on the whole been successful. Certainly, the mathematics should not overtax any mechanistically-minded research chemist (contrary to the impression he might gain on casually leafing through it) and there are many useful hints and practical comments hidden (the correct word, unfortunately) throughout the book.

Where I would be a little less than fulsome in praising the book is for its comprehensiveness. Although his choice will certainly not be universally applauded, it seems to me that Dr Bernasconi has quite properly and freely exercised his author's discretion on the topics for inclusion. Presumably, this is the reason behind his implication (with which I would not agree) that this is not a book for the physical-inorganic chemist. **D. N. Hague**

D. N. Hague is Senior Lecturer in Chemistry, University of Kent at Canterbury, UK.

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INTRODUCTION TO GEOLOGY: Volume 2 EARTH HISTORY

H.H. Read & Janet Watson

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This is the second volume of *Introduction to Geology*. Volume 1, *Principles*, was first published in 1962, with a revised second edition in 1968.

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Sedum of North America

Sedum *of North America, North of the Mexican Plateau.* By Robert T. Clausen. Pp.742. (Cornell University: Ithaca and London, 1976.) £42.25.

Sedum is the largest genus of the family Crassulaceae and consists of about 300 species, most of them in the north temperate zone but some on subtropical and tropical mountains. They are succulent plants, usually low-growing and small-leaved, and on account of their general easiness of cultivation and propagation and their numerous small starry flowers they have long been esteemed as rock-garden plants. Their garden value led an Irish botanist, Robert Lloyd Praeger, to publish in 1921 an account of the genus *Sedum* as found in cultivation (*J. R. Hort. Soc.*, **46**, 1-314), describing and illustrating 151 species, still a most useful work. A Swedish botanist, Harald Fröderström, published between 1930 and 1935 a survey of the genus as a whole in *Acta Horti Gothoburgensis*, distinguishing 327 species. Neither competes in bulk and wealth of detail with Robert T. Clausen's *Sedum of North America*, which devotes 742 pages to some 90 species, only 36 of them native. Unlike other workers on the genus, which he has studied intensively since 1935, Professor Clausen has been able to examine wild populations of almost every North American species and has supplemented his field surveys by experimental culture at Cornell University. Thus he has been able to separate environmental modifications from genetically fixed characters.

The result is this comprehensive monograph admirably illustrated with various sketch maps and with line drawings of all the species by Elfriede Abbe, who earlier provided those in Clausen's *Sedum of the Trans-Mexican Volcanic Belt* (1959). That work set out to be "an exposition of taxonomic methods", not simply a concise working account of 28 Mexican species, and provided a prolixity of detail irrelevant for almost all its users. In *Sedum of North America*, because the area covered is about 216 times larger (though with almost the same number of species), the same treatment has resulted in this huge and expensive work. It raises the question as to whether the printing (as distinct from the research and recording) of so many measurements in the diffuse paper-covering style of this book is justifiable nowadays, since the information relevant for most purposes could have been presented in a volume of less than half the size and half the price. It thus suffers from a defect produced by its major virtue. Having worked so long, so thoroughly and so successfully at understanding these plants which can indeed only be properly evaluated from living material studied both in the wild and under cultivation, Professor Clausen has evidently felt he must publish everything, an honest enough procedure but costly. Thus his account of *Sedum spathulifolium* occupies 35 pages; he nevertheless leaves somewhat uncertain Praeger's var. *purpureum*, of which he has seen no material, although this is still cultivated in England.

For anyone interested in the *Sedum* species of North America, their characters, population variation, nomenclature, distribution and identification, this work will be indispensable, which makes its high cost all the more regrettable. Likewise regrettable is the lack of attention to page headings; instead of the verso heading throughout the book unnecessarily proclaiming that it deals with "Sedum of North America", it could more usefully have given the chapter heading, while the recto heading then named the species dealt with beneath, which is often not evident. One hopes that some day Professor Clausen will produce a convenient illustrated handbook to the genus *Sedum* in America as a whole, summarising concisely the results of his many years of painstaking enquiry. **W. T. Stearn**

W. T. Stearn is Honorary Botanical Curator of the Linnean Society, and was formerly a Senior Principal Scientific Officer at the British Museum (National History).

Statistical mechanics of liquids

Theory of Simple Liquids. By J. Hansen and I. R. McDonald. Pp. xv+395. (Academic: London and New York, 1976.) £14; $30.65.

RECENT years have shown an upsurge of interest in the statistical mechanics of liquids, which has been marked by a number of important advances. The advance has been continuous since 1957; the previous history of the subject had been a series of fundamental advances, with long gaps in between.

This book is a timely and balanced survey of recent developments, shows how they are related to the older work, and explains the relationships between the various theoretical approaches. The year 1957 showed that the task of evaluating the correct consequences of an assumed intermolecular interaction function was within the power of large computers and this has stimulated a great deal of further work. One good feature is a special chapter describing techniques of these computer calculations.

The days of analytical results in this field are, however, very far from over, and these approaches are described in chapters 4, 5 and 6. Basically what has happened is that the fundamental advances made by Kirkwood (the feasibility of an integral equation approach) and by Mayers (the diagram expansion technique) have been systematically followed up and improved. It has also been found possible to develop perturbation theories analogous to those of quantum mechanics and celestial mechanics. These three chapters provide a connected account of these developments, contain very little repetition and are a pleasure to read.

Some workers consider equilibrium statistical mechanics to be difficult enough and fight shy of transport processes. A large amount of work has, however, been carried out, and is well summarised in chapters 7, 8 and 9, which the reviewer, largely an 'equilibrium man', found most helpful. In reading a published paper in this field it can be quite difficult to separate the formalism and the 'intelligent guesswork' or to see whether any difficulties of principle are being glossed over. These chapters will certainly be helpful to many workers, as will the account of generalised hydrodynamics, hard to find elsewhere except in original papers.

I was disappointed to see no mention of surface tension and related phenomena. One of the first definite results in liquid-state theory was Laplace's relation between surface tension and latent heat, and the subject was again opened up by the pioneer work of Kirkwood and Buff. Many of the published papers are, however, obscure and complicated; perhaps lack of space was the deciding factor. The final chapter, on phase transitions, seems to have suffered for a similar reason. The discussion of melting is as good as could have been hoped for in the present rather fragmentary state of knowledge but I would have liked to see a far more extensive account of the critical region. In particular, I would have liked to know the authors' own views on the following point. Why do scaling and renormalisation group methods provide good results despite the fact that they seem to use approximations just as drastic as those that vitiated older theories of phase transitions?

The book is well printed and produced but the price seems on the high side even at present levels. There is a valuable bibliography and a good index.
H. N. V. Temperley

H. N. V. Temperley is Professor of Applied Mathematics at University College of Swansea, University of Wales, UK

Drama of environmental physiology

Environmental Physiology of Animals. Edited by J. Bligh, J. L. Cloudsley-Thompson and A. G. MacDonald. Pp. viii+456. (Blackwell Scientific: Oxford, 1976.) Cloth £18; paper £9.50.

THIS book is a multi-author text for degree-level students who have at least some knowledge of physiology and ecology. Its scope is very wide for, as the editors state in a postscript (p434) "Between a mutation and the resultant marginal benefit detectable in a population there lies an enormously complicated drama acted out by individual organisms in an environment which is certainly indifferent and often hostile. Environmental physiology *is* this drama, and is thus the central subject of biology. It embraces the intracellular molecular biology which accounts for the occurrence of mutations, as well as classical unicellular and multicellular morphology, physiology, biochemistry and ecology. It demands the ability to think at different levels of analysis—at those of molecules, of cells, of organisms and of populations, because organisms adapt in different ways at these different levels".

The text is divided into four main sections, and there is also an editorial introduction (part I) and postscript (part VI). Apart from the editorial contributions, the main text consists of a short section (part II) of two chapters on the origin of life and the evolution of the oceans and atmosphere. This is followed by a major section (part III) of seven chapters on adaptive mechanisms which have resulted from long-term evolutionary changes. It includes excellent reviews on ionic and osmotic regulation in aquatic animals, water balance and excretion, gas exchange, and reproduction. Part IV on acquired adaptations contains a well-coordinated and stimulating series of chapters on non-genetic adjustment to change in environmental conditions. This is followed by a section (part V) on immediate homeostatic or sensory responses. The latter contains chapters on sensory physiology, buoyancy, colour change, and temperature regulation.

A feature of this book is the contributions by the editors to coordinate the text so that contributory chapters can be placed clearly into the context of the framework of the book as a whole. It is a praiseworthy attempt to overcome some of the problems associated with multi-author texts, and some of the prefaces and introductory chapters to each section are quite outstanding. Chapter 11 introducing the concept of acclimatory adaptation and chapter 16 on rapid responses of organisms to environmental change, are especially clear in introducing later chapters in each section. Other chapters are, however, less successful in this respect and are in places even slightly ludicrous in attempting to unite environmental concepts with those of physiology. After identification of the principal terrestrial environments in chapter 6, for example, the reader is informed (p102) that "The topography of the land and variations of its climate represent the same part in the make up of the environment, figuratively speaking, as morphology and physiology do in the life of an animal (12). Thus irrespective of climate, the mammalian fauna of desert, savanna, steppe, tundra and high plateaux have certain features in common". I was unable to fully understand either the precise meaning of the analogy attributed to Haviland (1926), or the logic of its relationship with the statement that follows it.

It was a little disappointing to find that there was no chapter on sense organs in the section on evolutionary aspects, and that no detailed consideration was given to topics such as accoustic interactions between bats and their prey in chapter 17, a topic which would surely have fitted in well with the concepts underlying the theme of this book. It was also slightly surprising to find in the chapter on locomotion that there were no diagrams of how fishes, quadrapeds and flying animals actually conform to Newton's three Laws of Motion, despite the excellent prelude to chapter 8 stating these as underlying principles. Perhaps these were assumed to be well-known from other texts, but it would surely have been well-worth illustrating them in a comprehensive book designed for the undergraduate level.

The contributions overall are of very high quality, and are remarkably free of mistakes. The book has been well compiled and conscienciously edited so that, in general, individual chapters are well-balanced reviews which fit the stated theme of the book. The continuity is greatly aided by the editorial contributions, some of which are exceptionally stimulating. This book will be a welcome addition to the shelves of the physiologist and ecologist. It is enjoyable to read and is to be highly recommended for courses in environmental physiology at university level. **R. C. Newell**

R. C. Newell is Professor of Environmental Biology at the Institute of Biology, Odense University, Odense, Denmark.

Nematodes

The Organization of Nematodes. Edited by N. A. Croll. Pp. viii+439. (Academic: London and New York, 1976.) £15; $32.75.

THIS multi-author volume contains fourteen chapters which describe various aspects of the structure, physiology and biochemistry of nematodes. The controversial topic of nematode taxonomy is discussed in the first chapter and is followed by chapters which review, to a greater or lesser extent, energy metabolism; the functional organisation of the head; the development and organisation of skeletal structures; sense organs and their secretions; nitrogen excretion, osmotic and ionic regulation; nematodes as models for ageing studies; respiratory physiology; hormones; neuromuscular physiology; the mechanics of feeding; behavioural coordination; the use of mutants to analyse the sensory system; and survival strategies.

The individual chapters of this book are of a high standard, and some are excellent. In a book with this title, however, one would expect complete coverage of all fields of the organisation of nematodes, but the alimentary system, with the notable exception of the oesophagus, and the reproductive systems, with the exception of the spicules and the eggshell, are not included; yet these are major systems in nematodes.

The reviews are also not grouped in a logical manner. This may be deliberate policy to emphasise the individual nature of each chapter, but one would, for example, expect to find energy metabolism, respiratory physiology and nitrogenous excretion dealt with in adjacent chapters. There is also overlap of subject matter in several chapters. This is inevitable in a multi-author volume, but sense organs, for example, are described in chapters 3, 5 and 13, with no cross references. It is unfortunate that the authors of these particular chapters were apparently not given access to each other's manuscripts. Several chapters are comprehensive reviews of the subject, whereas some others deal with a smaller number of examples or topics in greater depth. Some chapters include important work which has not previously been published. It is a matter for regret that the plates, especially the electron micrographs, do not occupy more of the page, as certain details have been lost as a result.

This book is a significant contribution to the literature on nematodes, and will be extremely useful to those who teach and carry out research on these important animals. It will also be heavily used by students who wish to pursue, in depth, the various topics dealt with in the book. **D. L. Lee**

D. L. Lee is Professor of Agricultural Zoology in the Department of Pure and Applied Zoology at the University of Leeds, UK.

HYPERTENSION

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edited by W. DE JONG, A. P. PROVOOST *and* A. P. SHAPIRO

PROGRESS IN BRAIN RESEARCH, Vol. 47

1977. about 380 pages.
US $ 57.95/Dfl. 142.00. ISBN 0-444-41534-3

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1976. xviii + 182 pages.
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WADDINGTON, C. H. Tools for Thought. Pp.250. ISBN-0-224-01077-8. (London: Jonathan Cape, Ltd., 1977.) £5.95 net.

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. There is a Reader Enquiry Form on page xxi.

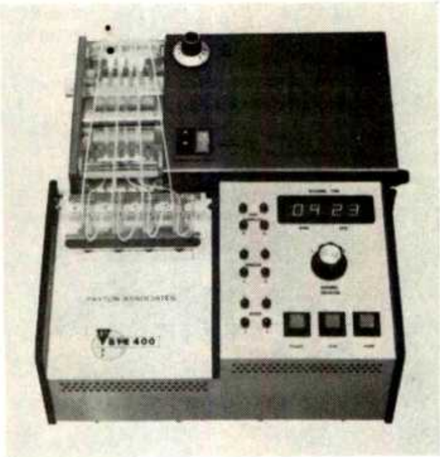
HPLC Column Injector System. Shandon Southern. The system comprises an optimised septum injector or an adaptor allowing the use of other injectors, a range of columns up to 8 mm bore, and a packing tube for slurry packing. These are designed as a unified system and combine the high efficiency of syringe injection with a standard of reproducibility normally associated with valve injection systems. Both analytical and small scale preparative chromatography are possible.
Circle No. 33 on Reader Enquiry Form

High-Resolution Underwater Detection System. Marconi. A digital sonar navigation, positioning and detection aid for the location, tracking and recovery of underwater objects such as wellheads, pipelines and divers known as Marfix, this portable acoustic detection system employs a new method of sonar electronic scan. Marfix comprises three main units: a processor, a display and a portable transducer which can be mounted on most vessels to scan either horizontally or vertically.
Circle No. 34 on Reader Enquiry Form

Digital readout refractometer. Anacon. The Model 42 provides instantaneously a digital LED display of refractive index and sample temperature. The readout can be calibrated in refractive index, percentage, degrees Brix or any other relevant unit and produces a repeatability of ±0.0002 RI. Provision of external water bath connections allow sample temperature control. Size: 13×6×10 in; weight 15 lb.
Circle No. 35 on Reader Enquiry Form

Automatic audiometer. B & K Laboratories. The Type 1800 Audiometer will provide extremely accurate and reliable automatic recordings in less than 10 min per individual, is for Bekesy type audiometry and provides a series of pure tone signals at seven different frequencies from 500 to 8000 Hz, either pulsed or continuous.
Circle No. 36 on Reader Enquiry Form

In vitro **Bleeding Time Recorder.** Payton Associates. The BTR–400 replaces the traditional *in vivo* method eliminating some of the limitations and undesirable aspects e.g. trauma, repeated incision, and scarring. It also minimises the risk of infection. Simultaneous determinations of up to four different blood samples are possible. Blood is pumped at a constant rate through tubes, near the end of each of which is a minute orifice. Drops of blood fall through a photo-sensitive detector and this information is translated into bleeding time, stored in a memory module, and displayed via a 4-way selector switch on a digital (LED) counter. The Payton system also facilitates mixing experiments and provides ready access to the hemostatic plug for further study.
Circle No. 37 on Reader Enquiry Form



Payton BTR-400

Minature cryogenerator. Pye Unicam. The type MC80 Mini-Cooler is an extremely compact, lightweight cryogenerator able to produce temperatures down to 80 K within 15 min.

The MC80 measures 38 cm high and weighs 6 kg. Operating on the Philips–Stirling principle,. the MC 80 produces a cold output, approximately liquid nitrogen temperature, at a copper 'cold finger' normally situated inside a transparent bell-jar vacuum head; there is provision for pneumatic lines and electrical connections to be made through the vacuum wall allowing observation of experiments carried out in the bell-jar.
Circle No. 38 on Reader Enquiry Form

Resonant optical scanners. Techmation. Operating on the oscillating torsion bar (bearingless) principle, these motors exhibit an extremely long mechanical life and are designed for use in applications where a single frequency between 100 Hz and 2 kHz, fixed scanning angle and extreme reliability are required. They show a high order of amplitude stability (0.6% per °C) and are supplied complete with precision mirror.
Circle No. 39 on Reader Enquiry Form

High versatility SEM/X-ray system. Philips. A high-performance scanning electron microscope PSEM 500 interfaced with a fully-focusing wavelength dispersive X-ray spectrometer, the PSEM 500X, not only permits analysis of X-rays from $Z=5$ to $Z=92$ but is the first such system to use the vertical spectrometer orientation—a configuration which offers the highest mechanical reproducibility—without the need for an optical microscope. Instead, a new standard in specimen height accuracy and reproducibility is achieved via the unique, fully-eucentric goniometer coupled with its 4,6 mm fine Z-adjustment (in 0,2 μm steps) and the large (210 mm) Rowland circle of the spectrometer. An extremely high-vacuum system (10^{-6} torr, inclusive of the spectrometer assembly) vastly improves practical light-element detection. Thus, absorption of 'soft' X-rays from light elements, due to specimen contamination, is seldom encountered. The same vacuum system, without modification, allows operation with optional higher-brightness sources—a particularly valuable facility for excitation of X-rays from light elements. The standard 50 kV electron optics of the PSEM 500 is ideal, when using an energy dispersive X-ray detection system, for unambiguous excitation of K-line X-rays from heavier elements. The larger excited volume of X-rays, when using 50 kV electrons, can also facilitate easier trace-element detection. The energy dispersive and wavelength dispersive spectrometers can be in operation at the same time, thus giving an optimum choice of the characteristics of each type of spectrometer.
Circle No. 40 on Reader Enquiry Form

CO_2 Incubator. Assab. In the T304GF, temperature is controlled within the range +25 °C to +75 °C to a tolerance of ±0.3 °C throughout the cabinet. The CO_2 level, ranging from 0 to 25%, is continuously analysed and controlled through the use of a thermal conductivity sensor which responds rapidly to changes in CO_2 as small as 0.1%. This system ensures the most economical use of CO_2 and rapid recovery of the preset level following door opening. Humidity is produced by the controlled recirculation of the cabinet CO_2/air mixture through a water bath. A polymer foil hygrostat, accurate to ±2.0% RH, senses the relative humidity and operates the circulating pump accordingly. Both temperature and Co_2 control centres are solid state plug-in units which can be easily replaced. Controls are provided to set required levels which are displayed on digital meters. If required, visual or audible high/low alarms can be specified for temperature and CO_2 functions. Dimensions: 800×600×600 mm.

Circle No. 41 on Reader Enquiry Form

Scanning electron microscope. Philips. The SEM 501 has a guaranteed 70 Å resolution which can be consistently achieved and often exceeded and uses a three lens optics achieving a wide range of illuminating conditions. Its illumination efficiency, smallest spot size of 40 Å and low magnetic field at the specimen all combine to give high resolution images. Specimen positioning, at any magnification within the standard ×4 to ×160,000, is easily attained. The SEM 501 incorporates TV rate imaging, 30 kV electron optics, micron marker, independent number of lines and line time selection, high resolution photomonitor, digital display of magnification, *Y* modulation, a modular specimen rotation unit.

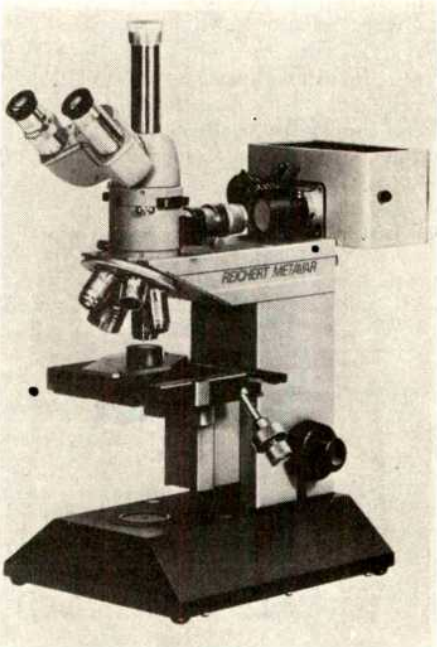
Circle No. 42 on Reader Enquiry Form

Electronic motionless switch. Siemens. A reliable, long-lived piezo-electric motionless switch for the direct control of ICs. The fully encapsulated switches can be used separately or grouped to construct switch panels and can be operated off symmetrical or asymmetrical voltages between 4 and 30 V, generates a current of 60 mA (adequate for miniature relays) for roughly 0.3 s under a brief pressure of around 150 g. In contrast to inductive proximity switches, this switch must be actuated by a defined pressure. A red, green or yellow light-emitting diode under a cap, which can be removed for the insertion of inscriptions, indicates the switch status

Circle No. 43 on Reader Enquiry Form

Metallurgical microscope. Reichert-Jung. The MetavaR microscope has a series of variants which enable all known metallurgical examination techniques to be undertaken and uses an identical stand throughout the range but alternative optics and illuminators depending on the technique. The incident light equipment is available in three options either for bright field polarisation, dark field or interference contrast. They are easily and accurately fitted or interchanged between stand or viewing tube. The S is for polarised light and bright field examination, the U is for examinations in bright field, dark field and polarised light; and the IK is for interference contrast, polarised light and bright ground examinations. Supplementary equipment for the series includes dual viewing projection screen, camera systems and drawing apparatus.

Circle No. 44 on Reader Enquiry Form



Reichert-Jung MetavaR metallurgical microscope

Uric Acid Clinical Diagnostic Kit. Pierce Chemical. The kit uses a unique method for quantative serum uric acid determination. Its direct serum procedure eliminates deproteinisation, and its unique two-tube method eliminates interferences from common drugs and native serum constituents. Absorbance values are linear to 20 mg dl^{-1}. The test can be run in a little over 35 min and colour development takes place within 15 min at room temperature. The final colour is stable for 90 min. The uricase is stable refrigerated; all other sonstituents are stable at room temperature.

Circle No. 45 on Reader Enquiry Form

Automatic sampling system. Hewlett–Packard. Batches of up to 60 samples can now be analysed automatically in sequence, and in replication if desired, by HP1084A and HP1082A processor-controlled liquid chromatographs. Only the sample amount injected is consumed by the system, making it possible to use micro-vials where only small samples are available.

A detachable unit containing 60 glass vials is located on top of the liquid chromatograph. A variable-volume sample injector, a standardd part of the chromatograph, then samples each vial in numerical sequence. The injection volume can be pre-set manually at any point between 10 and 200 μl.

Circle No. 46 on Reader Enquiry Form

Helium liquefier refrigerator. Sulzer. The Turbocryofridge TCF100, has a choice of two oil-free compressors, enabling a large number of plant variants to be achieved. By using the larger compressor and the cold box to its full extent with liquid nitrogen pre-cooling, it is possible to increase the minimum capacity of 10 l He/h (40 W at 4.5 K) to 80 l He/h (180 W at 4.5 K). For the generation of the necessary refrigerating capacity, the cold box is equipped with one or two turbo-expanders, each with dynamic gas bearings which operate at room temperatures. Braking is by a direct-coupled, single-stage centrifugal compressor.

Circle No. 47 on Reader Enquiry Form

TV attachment for light microscopes. Teletronics. A new closed-circuit TV attachment for multi-purpose microscopes, enables many people to view the same microscope slide simultaneously. The new system comprises a mini-CCTV camera, a 9-inch black-and-white TV monitor, and an attachment to link the camera to the microscope. Suitable for use with routine/research/biological microscopes having a sleeve of 23.2 mm inside diameter, the system gives users several options: camera-to-monitor cable distance can be from 1.5 to 100 m; the high-resolution 9-inch monitor can receive input from up to three cameras; and the monitor can be augmented by up to 10 additional monitor screens.

Circle No. 48 on Reader Enquiry Form

Automatic Colony Counter. Oriel Scientific. The Lab-Counter is an automated system giving fast and accurate counts of various types of samples both translucent and opaque. More than 700 samples per hour can be counted. The following samples can be taken by using the appropriate sample holder: microscopic slides, petri dishes 50–100 mm, tissue culture flasks, photographic films, filter dishes or photographs.

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Why believe all our claims for the new SEM 501?

The new SEM 501 gives you better micrographs, consistently, more conveniently than with any other competitive scanning electron microscope.

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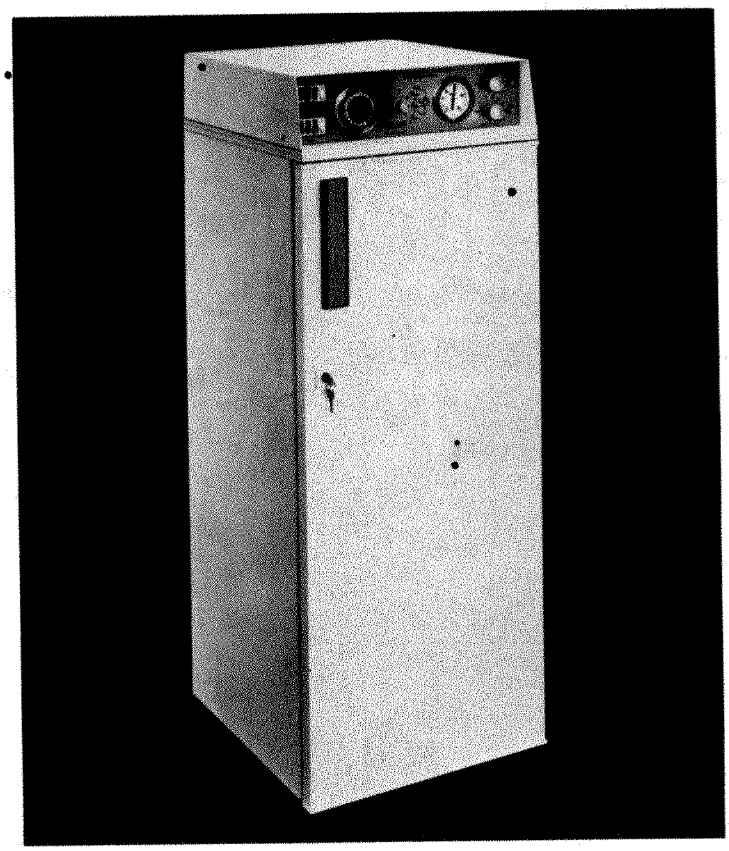
Circle No. 06 on Reader Enquiry Form.

Gallenkamp

Cooled Incubators -dependability



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Circle No. 07 on Reader Enquiry Form.

nature 14 April 1977

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The quiet side of technology

How many people understand what the state does on our behalf in prisons, in conflicts such as that in Northern Ireland, or in the murky world of demonstrations, pickets and strikes? And how well aware are scientists and technologists of the uses to which the armed forces, the police and the prison service put science and technology in pursuit of domestic policies? There is general and widespread ignorance of these subjects, and so the appearance of *The Technology of Political Control,* by Carol Ackroyd, Karen Margolis, Jonathan Rosenhead and Tim Shallice (Penguin £1.25), should have been welcomed as a guide to an uncharted area. But the book, which stems from investigations organised by the British Society for Social Responsibility in Science (BSSRS), is too ill-focused, full of ideology and lacking in constructive thinking to have anything like a major impact on the scientist who knows little and wants to know more.

The authors themselves write that their approach 'may seem a curious amalgam of technological exposé and political analysis' and indeed it is. The first half of the book is devoted to a rambling analysis of the steps by which Britain could move towards being a 'Strong State', obsessed less with the threat from without than with that from within as it lays plans in anticipation of social conflict. Such a strong state would have learned a lot from the Northern Ireland experience and would practise the sort of low-intensity operations described by Frank Kitson in a book of that name. The army, more closely integrated with the police, would play a significant role in putting down insurgency.

If you believe, as do the authors, that the major function of the British legal system is to protect property, that the events of the twentieth century are increasingly posing the question of socialism or barbarism and that any new working class revolution will have to ensure that ultimate power resides with the working class, then you will probably accept their analysis. A less class-conscious view of the world would probably not reveal quite the same trends, but that is not the point. The point is, as the authors insist, that they are writing as 'concerned scientists and committed socialists'. The actual technology of political control is therefore at least as important.

The technology occupies the second half of the book and amongst other things includes phone-tapping, bugging, computers, sensing equipment, riot control techniques, method of psychological torture and misuse of psychiatry. It is described as a 'vast and grim catalogue available to Western governments'. But the detail contains few surprises. Indeed, it is one of the disappointments of the book that it draws so widely on newspaper cuttings and so little on detailed research by the authors. Given the grass roots contacts BSSRS has been developing in the past few years, such research surely ought not to have been impossible.

Take the example of the computer. The police and the army already use computers as data banks, of course, and these uses are described. It takes little imagination to spot that computers may have a broader role to play in stitching together low grade information: as one Pentagon enthusiast put it some years ago, given an adequate computer network in Northern Ireland, one could put all sorts of information together, such as that Mrs O'Reilly hasn't gone out for her daily walk to the baker's, and come up with suggestive patterns of use to the security forces. But if this is true and it has been done, it poses all sorts of questions about the privacy of the individual. The authors only very tangentially pick up this issue, either technically or in its implications, and give little sign of persistent enquiry. So it seems with many other technical issues.

To the authors of this book, modern technology in the service of the state's law enforcement agencies is clearly an affront because it is or will be used to keep down working-class-generated insurgency and revolution. But what do they say scientists should do? It is here that the book is at its weakest and briefest. The answer seems to be that they should work politically for the abolition of the capitalist state and the establishment of a workers' militia in place of the capitalist army.

Those who take a less extreme view will be more likely to say that many of the problems of technology and political control are not amenable to such neat solutions. In Northern Ireland there is now plenty of technology on both sides, none of it particularly palatable, and talk of working-class control is meaningless. In other less strife-ridden parts of the country the unspectacular recipe is eternal vigilance. Technology does indeed have its role to play in law enforcement, and law enforcement agencies would be remiss if they did not use technology to the full. But the question is at what place use becomes abuse, reasonable enquiry becomes harassment, monitoring becomes intrusion. This can only be solved on a case-by-case basis, with at least some sort of trust in political institutions. The real need is for scientists who are involved in military and police research to be able to speak up when they see the balance shifting in an unsavoury direction. ☐

Directing Cuba's science

Mahindra Naraine, recently in the Caribbean, assesses the achievements of Cuba's science policy

IN FEBRUARY of last year, Cuba proclaimed a new constitution and established a new governmental system which transformed its existing central administration: ten months later, in December, the Council of Ministers—effectively the new Cuban government—was appointed by the National Assembly. The country was no longer ruled by decree, and elections had been held. The changes, marking the fourth phase of Cuba's modern history (see box), are now having their impact on the country's science and technology policy.

The institutional and infrastructural framework that the new order supercedes had a chequered record. It was characterised by the conscious promotion of science and technology—training personnel, creating research institutions, establishing means of international collaboration and starting research projects. Thus, in 1962 a new Academy of Sciences was created. In conjuction with the Soviet Academy of Sciences it assessed the country's natural resources, and, had as its first priority the creation of a soils map and national atlas. Other institutes were also established: the Institute for Sugar Cane Research, the Meteorological Institute, the Institute for Scientific and Technical Documentation and the Institute of Nuclear Research. In addition, the Ministry of Industry decided to set up special institutes to seek immediate or long term policies for the use of natural resources; these included institutions for the study of sugar cane by-products, mineral research, chemical research, meteorological research, standards and metrology.

In 1965 the National Centre for Scientific Research (CNIC) was created to carry out research in several fields, especially in the biological sciences. Most of its work is basic research, and it enjoys close associations with the various institutions and with the University of Havana. But although a few coordinating bodies were established—Councils of Agricultural Research, Sugar Research, Geology and Geophysics Research and so on—there was still no central directing body for science and technology. By 1974 the Cubans felt that they had a 'critical mass' of personnel and resources oriented towards research and development and that their socio-economic development was solid enough as a base for further work.

Camera Press

There was good reason for this. The investment in the universities in the 1960s began to bear fruit in the 1970s with a growing volume of research and increasing manpower. There are now more than one hundred basic research centres of varying size including research institutes, experimental stations and specialised laboratories, in which there are over 21,000 technical workers (about 20% of them university graduates); expenditure in 1975 on the basic research centres was $75 million. In 1957 there were an estimated 4,000 engineers and scientists and 5,000 middle-level technicians; in 1975 there were some 30,000 university graduates, about half of them scientists and technologists. University enrolment has grown from 15,000 (1959) to 30,000 (1970) to 83,000 (1975); the plan envisages 140,000 in higher education by 1980. In 1974 the breakdown by disciplines was:

Science and technology	52.8%
Agriculture	16.3%
Medicine	18.3%
Education	1.8%
Economics	7.0%
Social sciences	3.8%

In 1958 only 11% of the students were enrolled in science and technology. According to Cuban figures, there are 20 graduates and technicians per thousand of population. This means there are some 170,000 technicians in the country.

Highest priority

The Cubans have given education the highest priority, spending $874.6 million in 1975. The 1976–80 plan provides for 1.7 million in primary school in 1980, and 1 million in secondary school. There are four universities, and in the different provinces there are also 'university colleges' which are attached to one of the universities. 'University units' are centres of production, services and research where workers can carry out productive activities and take classes. University research is to a great extent carried out in teaching departments as an integral part of the students' science and technology training and the education of the staff. Some faculties have developed centres around specific topics, where teachers are assisted by researchers working in nearby schools and where postgraduates are trained. Some centres, for example the Centre for Digital Research and the Institute of Animal Science, do only research and postgraduate training; this type of centre is not directly dependent on the faculty or schools, and aims to train quality personnel and develop research.

With such advances, it was by 1974 time for more changes. In June of that year the government decided to establish a National Council of Science and Technology (CNCT) to manage the country's science and technology policy. Central management was deemed necessary to avoid publication, reduce cost, manage international collaboration and to coordinate the work of the various institutes with socio-economic development. Indeed, in establishing CNCT it was explicitly stated that a national science policy was a political not a technical decision.

CNCT was set up as an organ of the Council of Ministers with its President being the Vice-Premier of Education, Science and Culture. One of its vice-presidents was the President of the Academy of Sciences. The immediate task of CNCT was to draw up the national science and technology policy; once this was approved by the Council of Ministers, it was expected to control the execution of the plan and to propose practical application of the results obtained. The CNCT itself, which did not administer any research and development centre, had three main parts:

1. Presidential Council (President, Vice-Presidents, Secretary General and Directors.)
2. Advisory Committee (Members of the presidential council; well known scientists from different organisations and institutions; technical ministers and or vice-ministers concerned with research sectors and sub-sectors of research departments in universities; directors of the major research institutions.)
3. Scientific councils (Set up according to the nature and needs of the managing bodies, they are intended to bring together the most famous specialists in the relevant discipline independently of the organisations where they usually work.)

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Close collaboration with the Central Planning Authority (JUCE-PLAN) and with other bodies was necessary to achieve proper integration of science and technological plans with the socio-economic and cultural development of the country and to ensure that the science and technology plan would be one of the categories of the national economic plan. The 1976–80 economic plan was approved at the first Congress of the Cuban Communist Party in December 1975, and has three aims: to increase and diversify agricultural production to make the country self sufficient in food; to increase industrialisation; and to increase the production of sugar and nickel to pay for imports.

In agriculture the main areas of expansion will be rice, vegetables, citrus, poultry, fisheries and livestock. Industrial production will be increased in all areas, particularly fertilisers, steel, cement, paper and agricultural machinery. It is planned to produce about 600 cane harvesters a year from a Cuban design; this will reduce the workforce in the sector from about 370,000 to 50,000 by 1980. A nuclear reactor with capacity 880 MW will be built with Soviet assistance; in 1975 Cuban electrical generating capacity was 6.5×10^{12}W (about 700 kWh per inhabitant) but this is expected to increase by 35% over the next five years.

In 1959 sugar constituted 80% of Cuban exports and it held a similar position in 1970. In the future it is expected that the extensive Cuban nickel deposits will take over the role of main exchange earner, hence the 300% proposed expansion in nickel production.

CNCT plan

The CNCT drew up a plan for 1976–80 to link up with the national economic plan, slowly integrating indigenous research with technology transfer. Technology transfer is considered by Cubans to be indispensable to rapid economic development, but for it to be effective it must be adapted to local conditions and that means a large research and development base is essential. The CNCT plan focuses on the exploitation of Cuba's extensive laterite deposits; the application of nuclear technology and solar energy; the development of the electronics industry (to produce 100,000 television sets and 300,000 radios per year) and the development of computing and information systems (Cuban designed mini-computers are now being manufactured with Japanese components—under a Soviet–Cuban agreement, Cuba will provide the Comecon countries with certain components); the use of local products; and agriculture,

Cuban chronicle

Over the past hundred years Cuba has undergone three major transformations, and the development of its science and technology policy has tended to reflect this historical development.

The second half of the nineteenth century, ending with the first US intervention from 1898–1902, coincided with the rise of Cuban national consciousness and the struggle against Spanish colonialism. The first Cuban Academy of Sciences was founded in 1860, immediately prior to the Ten Years War, by about thirty European-trained academics, many of whom made contributions to world science. Attempts to introduce scientific and technical methods to Cuban agriculture, then in a primitive state, were unsuccessful; colonial conditions meant the Academy was 'peripheralised', and new development stemmed from technology transfers rather than indigenous sciences. By 1895 there were 1,186 students in institutions of secondary education—0.7 per thousand of population; the subjects studied were mainly Greek, Latin and rhetoric.

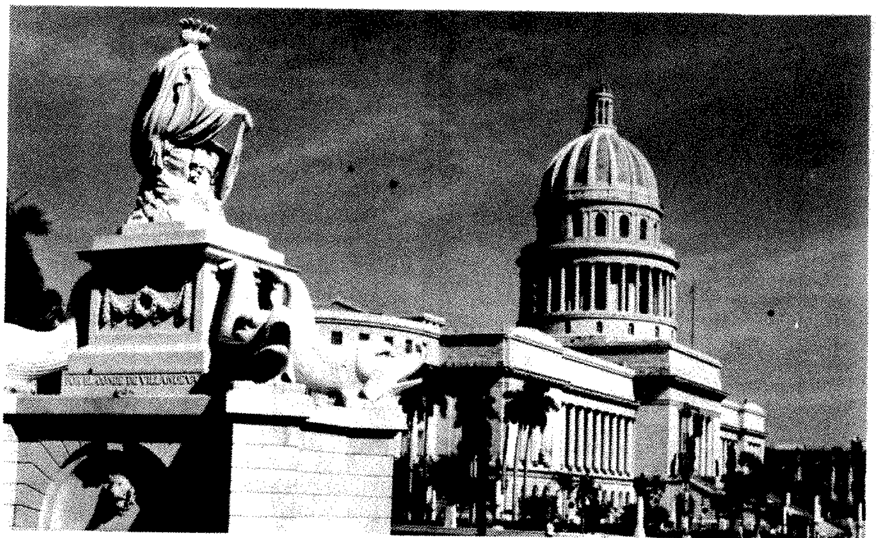
Cubans view the period beginning in 1902 and ending in 1958 as "the period of the neo-colonial republic marked by the presence of American imperialism." Local scientific activity was minimal and there were few research centres. The Academy of Sciences suffered a slow decline: by 1959 its yearly state budget was $600 and it did not possess a librarian. It was attached to the Ministry of Justice, while the Geographical Society of Cuba fell under the Ministry of State and the National Observatory was part of the Ministry of Sea and War. By 1958 15,000 students were enrolled in higher education, with 11% studying science and technology. About 60,000 students were in secondary education, representing about 10 per thousand of population. American missions in 1937 and 1950 commented on the paucity of technicians, the scientific and technological backwardness of the country and especially in the sugar industry, and the lack of facilities for research at the University of Havana.

The period was one of great political instability and culminated in the Batista dictatorship. The plantation economy, dependent on sugar for more than 80% of exports, was heavily reliant upon the US economy both in terms of trade and direct investment. The urban/rural dualism intensified, and by 1958 Cuba had half a million unemployed and two million illiterates and semi-literates in a population of 6.5 million. The period is seen as one of increasing cultural domination with continual attempts to belittle Cuban achievements. The example is cited of the recognition given to the American Dr Walter Reed as the discoverer of the carrier of yellow fever; in Cuba the Cuban Academician Dr Carlos Finlay is considered to have deciphered the problem.

The successful revolution of Fidel Castro and his followers in 1959 marked the start of Cuba's third period. Influenced by Soviet industrialisation, Castro and particularly Ché Guevara felt that a similar programme was necessary for the Cuban revolution to succeed. Science and technology were therefore recognised as critical factors in social and economic development, and in spite of a critical resource shortage, human and material resources were allocated for the creation of research centres to meet the requirements of the development plan.

The development plan which emerged for 1961–63 followed eighteen months characterised no less by chaos and government measures to gain control over all aspects of Cuban society, than by a programme of agrarian reform and a campaign to eradicate illiteracy. It was drawn up by Guevara and was intended to transform the Cuban economy from an agricultural to an industrial structure. It was expected to accelerate development on many fronts by means of import substitutions with emphasis on heavy industry. The programme exceeded Cuba's technical, administrative and resource capabilities; it ended as a failure.

The second growth strategy from 1964–75 reoriented previous plais, with their emphasis on agriculture, to centre on sugar and exports. Industrialisation remained the long term objective, but it was to be a gradual process with immediate expansion on a few fronts, such as cement manufacture, fertilisers and steel. Consequently, the science and technology infrastructure continued to expand, particularly in the training of personnel and in the establishment of research centres (see main story).



Cuba's Academy of Sciences, formerly the State Capitol

Barnaby's

livestock and fisheries. Over 100 problem areas have been identified for research and development activity.

Under the most recent changes, however, the National Scientific and Technological Council (CNCT) has disappeared. Its duties have been taken over by the State Committee for Science and Technology (CECT), whose head is a member of the Council of Ministers. This committee is the agency in charge of directing, coordinating and supervising the implementation of state and government policy in science and technology. It has to advise the government, prepare science policy, supervise research, establish research centres, and manage the science and technology information system.

The Academy of Sciences is now classed as an institute that is a central state agency, but its director will not be a member of the Council of Ministers. It has been charged with the drawing up, supervision and implementation of basic research and with being the link with foreign organisation. The various other technical ministries, covering, for example, the chemical industry, and mines and geology, will continue to have their own laboratories and do their own industrially oriented research. The work of all such ministries, however, will be coordinated by the CECT, which will also link up with the Academy of Sciences, the Ministry of Higher Education and the Ministry of Economic Collaboration.

Growing role

Cubas' international role is now growing; it is unusual to find a country so little endowed with natural resources such a large source of aid. Cuba's aid essentially comes from the technical expertise that is the result of its massive investment in scientific and technological education. The success has been little short of phenomenal, and it is estimated that there are more experts in the developing countries from Cuba than from any other country. But it is well known that Cuba has not achieved this by its own means. Since 1960 it has been drawn into the Soviet orbit; in 1970 the Soviet–Cuban Commission for Economic, Scientific and Technical Cooperation was formed, and in 1972 Cuba joined Comecon. Cuban development has been underwritten by the Soviets to the estimated sum of $5 billion since 1959. The full contribution of the Comecon countries is hard to assess, for apart from credits they provided expertise in the early years and also trained Cuban personnel. In 1969 as much as 20% of the scientific and technical staff engaged in research and development in Cuba were foreign but this manpower assistance declined as the Cuban investments in education began to produce results around 1970.

An important instrument in Cuba's new international role is the State Committee for Economic Collaboration (formerly the National Commission for Economic, Scientific and Technical Collaboration). In coordination with the State Committee for Science and Technology, it plans and administers foreign technical aid. As the relentless industrialisation drive continues, Cuba's voice is growing more important in the 'North–South' dialogue. Along with India and Yugoslavia she has been one of the leading advocates of greater trade between the underdeveloped countries. For a country that in the next decade expects to be the most industrialised of that group, it sounds like good business. Mexico and Cuba have already designed sugar mills and equipment for sale to the developing nations.

How far Cuba will be able to continue this programme and supply these countries is uncertain. What is certain is that a massive investment in science and technology has meant great political and economic gains. Castro was indeed prophetic: "The future of this country," he said in 1960, "must be the future of men of science." ☐

USA

Feasible, sound and attractive

A new report on the prospects for solar energy was published last week. Colin Norman reports from Washington

GIVEN sufficient political and economic support, solar energy technologies could supply as much as 40% of the world's energy needs by the turn of the century, and a startling 75% by the year 2025, according to a report published last week by the Worldwatch Institute, a small think-tank concerned with global problems. Such a major shift from fossil fuels to renewable energy resources "would require an unprecendented worldwide commitment of resources and talent", but the social and economic benefits "would far outweigh the costs and difficulties", claims Denis Hayes, who wrote the report[*].

Though a multitude of enthusiastic articles and studies have been written in the past few years to extol the virtues of solar power, the worldwatch report is already attracting considerable attention. For one thing, it was published while the Carter Administration was in the midst of putting together a long-awaited energy policy for the United States, a policy which is expected to place increased emphasis on renewable energy resources. It also breaks new ground in analysing the worldwide potential for solar power, while most other studies have concentrated on solar prospects in a single country. And its upbeat conclusions are also tempered by some realism about the political obstacles along the road to a solar powered world.

Hayes begins by noting that a transition from oil and gas to alternative power sources is inevitable, given the fact that production of oil and gas is expected to peak in the 1980s and that the price of those fuels is already beginning to increase by leaps and bounds. He argues that a switch to coal to provide energy needs over the next 50 years would result in "an absolutely intractable problem" of massive increases in atmospheric carbon dioxide, that a switch to nuclear power would require building so many nuclear reactors and recycling so much plutonium that "such a prospect cannot sanely be greeted with equanimity", and that fusion power is a long distance, unproven technology. That leaves the solar options, which Hayes defines as wind, falling water, biomass and direct sunlight.

Though Hayes is quick to acknowledge that most solar technologies are hard put to compete economically with conventional fuels at current prices, he argues that many alternative power sources have environmental costs and hidden subisidies which are not usually included in economic comparisons. As those costs are not internalised and as oil and gas prices spiral upwards, solar energy will become increasingly attractive, Hayes suggests.

Straightforward comparisons between solar power and conventional fuels also neglect an important factor: solar energy is not just more of the same. "Most energy decisions are based on the naive assumption that competing sources are neutral and interchangeable", Hayes notes, but solar technologies offer a highly decentralised and flexible power source which, if widely used, would result in a very different world from one powered chiefly by large central generating stations running on coal or nuclear fission. Solar technologies, Hayes argues, "are more compatible than centralised technologies with social equity, freedom and cultural pluralism".

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[*] *Energy: The Solar Prospect* (Worldwatch Institute, 1776 Mass Avenue NW, Washington DC 20036, $2).

Hayes's basic message, therefore, is that a transition to a solar era "would be technically feasible, economically sound, and environmentally attractive". Furthermore, "the most intriguing aspect of a solar transition might lie in its social and political ramifications". A constant refrain running through Hayes's study is that many relatively unsophisticated solar technologies are already sufficiently developed to be put to use, either alone or in tandem.

On solar heating and cooling, for example, he argues that more attention should be paid to the thermodynamic efficiency of energy production and use, suggesting that it is "surely the height of thermodynamic foolishness" to use a nuclear reactor operating at thousands of degrees to run a water heater to heat bath water to 30 degrees. About a third of the energy now used in the world provides low-temperature heat, which solar collectors can already achieve in many parts of the world, Hayes claims. Moreover, if the lifetime fuels costs are properly counted in economic comparisons, he contends that solar heaters are already able to compete with conventional fuels.

Another promising renewable energy source is small hydroelectric plants which could easily be constructed to serve small villages. Even in the United States, Hayes reckons, there may be a as many as 50,000 potential sites available for hydropower plants as small as half a megawatt. There is also a large and relatively untapped source of energy from the production of methane from animal wastes, ethanol from fermenting plant wastes, and low-grade heat from biomass, Hayes suggests. A start has, however, been made on some of those technologies, particularly in India and China which are operating a substantial number of methane plants, and Brazil and Australia, where fermentation plants running on sugar cane and eucalyptus wastes are already in production.

As for more sophisticated uses of solar energy, such as large-scale generation of electricity, Hayes argues that although massive solar generating plants would hold considerable environmental advantages over conventional and nuclear power stations, they would also carry some of the disadvantages of inefficiency and centralisation. "A sensible energy strategy demands more than the simple-minded substitution of sunlight for uranium," he suggests. Photovoltaic cells, which convert sunlight directly to electricity, have the advantage of providing a decentralised power source which could be used in tandem with other small-scale generators such as windmills, Hayes notes, suggesting that photovoltaics "may offer the most exciting solar electric prospect".

Unfortunately, however, they also have a major disadvantage: their cost is at present prohibitive. Nevertheless, Hayes is optimistic. He notes in his study that the cost of photovoltaic cells has dropped from $200,000 per peak kilowatt in 1959 to about $13,000 today. If the trend continues, and if it is helped by economies of scale, Hayes reckons that the cost may drop to as low as $500 per kilowatt by 1985.

If the transition to a solar era is not made during the next 50 years, Hayes argues, "the roadblocks will have been political, not technical". With massive investments already made in coal and nuclear technologies, and a large gap between future energy supply and demand generally perceived by energy planners, the political roadblocks are formidable, however. President Carter's forthcoming energy policy, which is expected to be announced next week, is likely to provide a boost to both coal and light water reactors, though it is likely to pour some cold water on longer term nuclear plans. As for solar technologies and other renewable resources, it is expected to provide nothing of the support, and certainly nothing of the magnitude which Hayes calls for. ☐

Recombinant DNA bill proposed

THE Carter Administration last week finally proposed legislation to control all recombinant DNA experiments in the United States, after some three weeks of discussions between the Secretary of Health, Education and Welfare (HEW) and officials from the National Institutes of Health (NIH) and the White House. Although the final version of the bill conforms closely to a draft version proposed last month by an interagency task force, it differs in one key respect: it would allow state and local governments to establish controls on the research which are more stringent than the federal regulations.

The bill was formally introduced into the Senate last week by Senator Edward Kennedy, Chairman of the Senate Health Subcommittee which will handle the legislation. Kennedy made clear, however, that he doesn't necessarily agree with every word in the bill and in a statement he outlined a few reservations with the Administration's approach.

The bill would essentially require the Secretary of HEW to establish regulations to control recombinant DNA research in both public and private institutions. He would be required to issue interim regulations within 90 days and a final version within a year, after allowing suitable time for public comments and discussion. The interim regulations, at least, would probably be the guidelines issued last year by NIH. The bill would also require that all facilities housing recombinant DNA experiments be licensed by HEW.

There is also a provision in the bill which would empower HEW to send inspectors into laboratories the ensure that the regulations are being followed and violations could result in a fine of $5,000 per day. Particularly serious and wilful flouting of the rules could also result in a prison term, the bill states.

As for the provision allowing state and local governments to set stricter controls than the federal regulations, many scientists are now supporting federal legislation on the grounds that uniform national regulations would be preferable to a patchwork of local controls of varying stringency. The draft version of the Administration's bill would have accomplished that by simply making the federal controls paramount, but the final version would allow the states to add extra controls if they want to. According to Administration sources, the provision was changed so as not to throttle local debate over recombinant DNA but it also bows to the political reality that a move to pre-empt state and local control would be unlikely to be passed by Congress.

The focus of legislative activity has now shifted to Senator Kennedy's Health subcommittee, which held a public hearing last week. One provision which Kennedy may add to the bill is a requirement that the National Commission for the Protection of Human Subjects of Biomedical and Behavioural Research, which has been looking into a variety of biomedical issues for the past two years, be given the task of examining the long-term implications of genetic engineering.

Colin Norman

Gloom at the top

Details of Britain's Science Budget, were disclosed last week. Chris Sherwell reports

IF THERE is despondency in Britain's science community this week, no one should be surprised. There is gloom at the top. Details of the Science Budget given last week by the Advisory Board for the Research Councils (ABRC) covered allocations for the 1977–78 financial year just begun and guidelines for the four additional years to 1981–82. The figures are much as expected (see table), but then sliding down a curve is no more comfortable for being warned of it in advance, particularly when (as now) the low point remains invisible.

Outlining the trends, the ABRC chairman, Sir Frederick Stewart, said the 1977–78 Science Budget of nearly £249 million (1977 prices) represented a cut in real terms of more than 3% on the 1976–77 budget. The government's public expenditure White Paper published in February indicated a budget of £216 million for the year, but this was at 1976 prices. Both figures included an additional £6 million to cover the increased cost of post-graduate tuition fees in 1977–78, and this too tended to make the trend less steady than it actually was.

Discussing the guidelines, Sir Frederick drew on the White Paper's own emphasis on the provisional character of figures for the years 1980–81 and 1981–82 in describing them as "very provisional"; they were intended to provide each of the councils with a broad basis for planning. The guidelines did, however, represent a continuation of the protection of the "small and medium sciences" at the expense of high energy physics, astronomy and space.

Thus the Science Research Council (SRC) faces a 1.7% contraction annually, a cut which Sir Frederick said could amount to more than 20% over a five-year period with vexed problems like 'salary creep'—the consequence of maintaining ageing research staffs on graded incremental salary scales.

On the other hand, in line with the SRC's and the ABRC's previously announced intentions, support for engineering is to grow substantially, and is in fact the only part of the budget to show a marked increase. How much this increase will prove to be by the end of the period was uncertain, Sir Frederick said, but it "might amount to a 60% expansion". The SRC chairman, Sir Sam Edwards, stressed, however, that even a modest effort in favour of engineering would represent a large increase on the support that it received before. He was also quick to dismiss suggestions that the perceived need to support work 'relevant' to the country's industrial needs diluted the SRC's task of supporting basic research: the SRC's support for engineering, he insisted, was for basic research, just as it was in other fields.

Sir Sam described the reductions in support for big science as more serious than the figures suggested. Much of the SRC's expenditure went on international subscriptions, for example to CERN, and that meant that the effective decline in support for domestic activity might be more than 50%, not 20%. This, he said, would happen at a time when the pressure was on for Britain to participate fully rather than merely "tick over"; there were many projects which ought to be done "bigger, better and faster" than they were being done.

The position on international subscriptions clearly remains precarious. The SRC has the money to pay them this year, Sir Sam said, but if the pound collapsed again, "I don't know where we are"; the government had not given an open-ended commitment, and no bailing out could be guaranteed if that again proved necessary. As for the SRC's maintenance of a "minimum viable level of support" for big science—a concept outlined last year as the cuts really began to bite—Sir Sam confirmed that the big science community already believed that that level had been breached. The council's view, he indicated, was that it was extremely difficult to judge precisely what that level was.

Like the guidelines, the allocations of the 1977–78 budget reflect the policy of redeployment away from big science, though as usual the lion's share necessarily goes to SRC. Also given are figures for research commissioned by government departments under the Rothschild arrangements for applied research.

For the Agricultural Research Council (ARC) income from commissioned research, representing 55% of its total budget, will grow at 0.6%, but this will only contain the 'salary creep'. The Medical Research Council (MRC) faces more of a problem. As part of the general cuts in government expenditure the Department of Health has at short notice cut by 10% its commissioned research. The Natural Environment Research Council expects to have its funds maintained from the various departments (Agriculture, Industry, Energy, Environment), but anticipates cuts in the future. Against all this, Sir Frederick said that he saw no evidence that the spending departments were taking their funds elsewhere, a possibility Rothschild originally foresaw.

There is no doubt that the research councils face problems. Apart from the usual agonising difficulties of deciding priorities and, more notably, starting new projects, they agree that their main problem is nothing less than "keeping science alive" in Britain. In that they were echoing the views contained in the recent annual survey from the University Grants Committee (UGC), which with the research councils constitutes the Dual Support System for university research.

"We are deeply concerned", said the UGC, "about the deterioration of research capabilities in the universities . . . We will continue to watch the developing situation with anxiety". Those words might well have been repeated last week by the research councils for the whole of Britain's civil science effort. ☐

Science Budget: Allocation and Guidelines

	£ million[1] 1977–78	% Share	% p.a. 1978–79 to 1981–82
ARC	20.4	8.2	1.9
MRC	42.3	17.0	1.6
NERC	27.2	10.9	2.5
SRC	138.0	55.4	−1.7
SSRC	14.6	5.9	1.9
British Museum (Natural History)	4.2	1.7	0.0
Royal Society	2.2	0.9	1.0
Total	248.9	100	

[1] Figures are at 1977 prices. They exclude estimated receipts of £52.5 million from commissions from government departments (ARC £24.2 million; MRC £11.7 million; NERC £16.6 million)

BRITAIN

● Unsurprisingly, yet another view of the energy problem was heard at last week's 'Energy in the 80s' conference organised by the Institute of Chemical Engineers in Middlesbrough. It came from L. G. Brookes of the UK Atomic Energy Authority (UKAEA) —speaking in his personal capacity, of course—and it was aimed at undermining the 'conventional' view.

The conventional view, said Brookes, argued that the OPEC oil price rises have been a good thing, by inducing thrift and providing an incentive to develop other alternative energies, so that reduced demand and promotion of coal, nuclear power and alternative energies will close the energy gap. According to Brookes, however, price rises are not the solution to the problem: "they are themselves the problem".

His argument is simple. High energy prices have affected the economy adversely. That has hit the rate of growth of electricity demand, upon which the rate of nuclear power installation depends. That in turn has severely reduced the rate of substitution of nuclear for fossil energy. "It follows", says Brookes, "that a reduction in energy prices (or at the very least their stabilisation) [is] our number one energy policy objective".

Brookes' leap of logic is based on his view that uranium, coal and Middle East oil are the only large sources of low cost fuel, that uranium, used in breeders, is a greater source than all others together, and that nuclear power is the cheapest, safest and cleanest way of producing electricity.

Those with problems accepting nuclear power will be quick to question the thinking behind such arguments. But there will be others who will find some of Brookes' additional conclusions controversial as well. For example: high oil prices have actually lowered "the level of economic high spirits necessary for major investment in new energy sources", and her current "flirtation" with wave and solar power, which arises "to some extent from a misdiagnosis of the problem", may do harm if it is seen as an alternative to nuclear power. There is no reason to take it for granted, he adds, that the days of cheap energy are gone forever.

The Department of Energy's view is different, naturally. Last week it announced that the £1 million feasibility study of wave power it unveiled last year is to receive a boost following the encouraging developments experienced so far. Now a total of £2.5 million will be spent by October 1978, with the extra money going mainly to larger-scale studies.

● Smokers in Britain who have felt under increasing pressure over the past couple of years will have noticed the recent period of fits and starts experienced by Britain's cigarette industry. Measures to reduce the health hazard from smoking announced at the beginning of last month included a strengthening of the warning on packets, moves to extend public no-smoking areas and curb advertising, and the prospect of an end to high tar brands.

Close control of tobacco substitutes and additives according to guidelines from the Independent Scientific Committee on Smoking and Health (the Hunter Committee) was also sought, and at the end of the month the government gave the go-ahead to part-substitute cigarettes, the first of which may be on sale in July, subject to agreement on long term health studies. The Hunter Committee said it had decided to raise no objection to the carefully controlled use of two cellulose-based substitutes, Cytrel, made by Celanese Corp., and New Smoking Material (NSM), made jointly by ICI and Imperial Tobacco. The cigarettes will be mixed with about 25% tobacco, will carry the government health warning and will be no cheaper.

The price of cigarettes has since gone up, and the House of Commons Expenditure Committee on Preventive Medicine has recommended legislation against advertising and advocated dearer prices still, stronger health warnings and more non-smoking accommodation.

● The National Radiological Protection Board (NRPB), which last month published a review of its recent work over the three years 1974–76 (*The Work of the NRPB 1974–76*, HMSO, £2), published a paper last week on the radiation exposure of the British population (*Fallout in rainwater and airborne dust-levels in the UK for 1975*, NRPB-R49, HMSO, 50p). Based on the analysis for fission products and plutonium isotopes of rainwater and airborne dust collected during 1975, the results show average depositions of ^{137}Cs and ^{90}Sr in rain were less than 2% of peak values recorded in 1963 following a period of heavy weapons testing. Fallout during 1975 will contribute another 1% to the total dose people will receive.

● The departure of Francis Crick from Cambridge to the Salk Institute has directed widespread attention at academic salaries and the position of those who earn money abroad. So what are the circumstances of those at the top of the ladder?

Professors and their counterparts in MRC units are paid a minimum of £8,016 in 1976–77. There is no upper limit in universities, provided each university's average salary does not exceed £9,489. The MRC's Special Appointments Grade tops out at £10,200, though higher salaries are paid in respect of special service.

Five years ago the minimum was £5,376 and the professorial average £6,528; the cost of living index has since risen by at least 75%, while salaries have risen only 45% to 50%. Though everyone in this salary range has suffered substantial setbacks in real income, university and research council employees have suffered more than most. Many have been able to supplement their income by doing short periods of work abroad. Until 1974, this income was not taxed unless brought into the country, but in the past three years those who work abroad in a wholly separate job with duties entirely performed outside the country have been allowed only a 25% tax exemption of their earnings, regardless of where the money is kept.

The recent UK Budget has liberalised the tax position for many who work overseas under tax conditions less favourable than this, but has not made any further concessions to the category which includes visiting academics. The level of taxation on the 75% taxable will depend, of course, on the total sums earned in a year, but would typically run at a level from 45% if total taxable income in the year (including UK salary) was between £7,000 and £8,000, up to 83% for over £21,000.

IN BRIEF

Windscale inquiry dates

Mr Justice Parker, who was appointed along with two assessors to conduct the inquiry into British Nuclear Fuels Ltd's plans for an oxide fuel reprocessing plant at Windscale, is to conduct an informal preliminary meeting open to the public and press to discuss the programme and procedure for the inquiry. The aim of the meeting, on 17 May in Whitehaven, Cumbria, is to allow all concerned parties to make representations on these matters ahead of the main inquiry, which opens on 14 June also in Whitehaven.

Them and us

The difference in attitude towards foreigners between the general manager of R&D at ICI and the Chief Scientist at the Department of Industry (DoI) is that the former thinks of them as 'us' and the latter as 'them'. Duncan Davies, who made the transition from one to the other when he left ICI at the end of March, last week expressed his regrets at losing some of his former international involvement. Talking of British industry, he said that the 'entrance fee' for innovations—the initial investment to develop an idea into a marketable product—had increased by several orders of magnitude over the past twenty years. To ensure that it is recovered, better planning was needed at the initial stages of a project. Highly trained scientists and engineers, he said, should also be trained to judge the potential of an idea in the marketing as well as the scientific world before committing industry to a large investment which it might otherwise lose.

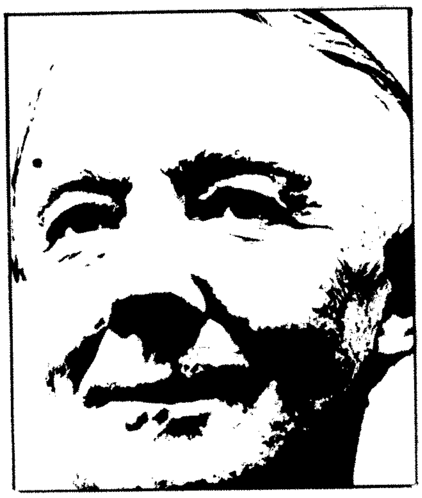
RGO exhibition

The Royal Greenwich Observatory at Herstmonceux has put together, out of its own resources, a small exhibition on the castle and its environment and the work done by the observatory. The castle already has numerous visitors to the Isaac Newton Telescope and to the spacious grounds; they will now be able to visit the exhibition as well.

Chemical hazards report

A report published recently by the Chemical Industries Association's Safety and Health Council (CISHEC) provides the industry with details of a technique to combat potential hazards in existing and new large-scale chemical processes. The technique, known as a Hazard and Operability Study, involves systematic study of every part of the proposed process and speculative consideration of the consequences of every possible deviation from the norm. Remedial action for every potential hazard is then noted. (*A Guide to Hazard and Operability Studies,* CISHEC, £4.50.)

Publish or perish



KENNETH MELLANBY

MANKIND has always suffered from disasters. The archetypal catastrophe was the flood, described in the Hebrew scriptures and in various middle eastern legends. Only eight individuals out of the whole human population were said to have survived to repopulate the globe with their progeny and that of the other animals saved from drowning in Noah's ark. Then in the second millenium BC the Aegean island of Santorini exploded, burying its cities in debris, and causing a tidal wave which may have ended the Minoan civilisation in Crete. This view was held by the leading Greek archaeologist, Professor Spirodon Marinatos. Shortly before his death in 1975 I heard him speak of his studies and demonstrate his excavations on Santorini. With all the signs of disaster around him he communicated his enthusiasm even when addressing a largely English-speaking audience in Greek; almost the only words I actually understood were *καταστροφη* and *κατακλασμα*.

No one else was left to organise an expedition to help in Noah and his family's rehabilitation, and the more fortunate populations on the shores of the Mediterranean were not in a position to feed and house any survivors in Crete. Today, however, with improved communications, the rest of the world soon knows of disasters in remote corners of the globe: funds are raised and expeditions with food, medical supplies and means to improvise shelter are rapidly dispatched to try to reduce the misery of the victims. It is sometimes ironical to note that there may for generations have been chronic shortages of all the necessities, with thousands of preventable deaths each year, in these same areas, and that world opinion has remained unmoved until a spectacular flood or earthquake has occurred. Then financial and material aid has poured in, making at least some of the population (perhaps the least deserving members) richer than ever before.

Aid for the victims of disasters has not always been appropriate in kind, nor has it always been administered efficiently. Various international agencies have built up a corpus of information, but this is not readily available to those wishing to help. For this reason the London Technical Group has launched a new quarterly *Disasters: The International Journal of Disaster Studies and Practice,* aimed at educating scientists and relief workers. It says it will describe both successful and unsuccessful experiments in disaster relief.

The first number reports on a UNESCO conference on the assessment and mitigation of earthquake risk. There are articles on the drought in the Sahel and on emergency shelters. Various useful equipment is described. Perhaps the most interesting contribution is a letter based on experience of the Skopje earthquake in 1963 in Yugoslavia, which shows how the local population, with the materials already available, produced effective makeshift shelters long before expensive and inferior emergency houses could be shipped from abroad. This is one instance of how aid can be misused due to the ignorance of the well meaning. If future issues can keep up this standard, the journal deserves to succeed.

Our attitude to disasters, which we share with the insurance companies, has curious theological implications. A colleague, a member of his parochial church council, was active and successful in raising money to repair a mediaeval church whose roof suffered in the gales of January 1976. At the end of the year, surveying the relatively favourable balance sheet, he informed his somewhat surprised rector that everything should be satisfactory for the next year or two, "unless we suffered from another act of God".

correspondence

Oklo today

Sir,—It is obviously of great interest to trace the fate of the fission products from the natural nuclear reactor at the Oklo River, Gabon (17 March, page 206), but if parallels are to be drawn with present day reactors and radioactive wastes, then one further fact needs to be stressed, namely that today's world is seething with complex living organisms. 1.8×10^9 years ago there were, in all probability, only comparatively simple organisms.

Today's organisms have very considerable powers of dispersal so that any radioactive chemical which enters one organism may be transported a considerable distance as it travels through the food chain; and higher organisms (mammals for example) are 1,000 times more sensitive to radiation than are the simpler forms of life (such as bacteria) which might well have existed at Oklo. To argue that since we, as living organisms, survived Oklo we can also survive comparable releases of radioactivity today is dangerously misleading. Of course life will survive the next few hundred years, but the question is at what cost to the particular forms of life that we consider to be important such as our own species.

Malcolm Edmunds

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Preston Polytechnic, UK

Scientists as administrators

Sir,—Your issue of 27 January, 1977 carries two articles which are complementary. The first is abstracted from Sir Hermann Bondi's broadcast and points out the very limited movement of scientists to administrative posts in the Civil Service and in industry in general; the second, by Kenneth Mellanby, provides the apparent paradox by emphasising that, in war-time, academics immediately turned to administrative tasks with great success. Mellanby goes on to ask why academics do not do this now.

In war, there was one clear objective—the enemy. The desired end was agreed by all, and a constant beacon to steer by. Not so now when national goals are confused and form the subject of bitter political wrangling and power-seeking. Yet, if, for example, the intensive cultivation of the Highlands of Scotland was essential for national survival, I have not the slightest doubt that the scientific ability needed to do this would flow as readily and effectively as it did to defeat the Nazis.

Sir Hermann points out that scientists as administrators must be seen to be badly needed. Rightly or wrongly the scientist is still often made to feel a poor relation of the arts man in respect of administrative ability, though not in organisations such as Unilever and ICI whose effectiveness as innovators is remarkable.

This will not be changed simply by altering the structures of science courses, but by a positive effort to encourage scientists to move to administration. I would like to see advertisements for administrators, stating that applicants with a scientific and technological background are preferred, and would be given the appropriate training if needed. After all, professors of science and technology and the heads of departments in government scientific institutes began life as rather shy, young scientists: most are now capable administrators and few would doubt their capacity for argument—written and spoken—sharpened and tempered as it is by the ceaseless battle with well-meaning but uncomprehending, non-scientific administrators.

P. J. Heald

Department of Biochemistry, The Todd Centre, Glasgow

Cutting out abbreviations

Sir,—Adrian Smith's suggestion (10 February, page 492) that abbreviated titles of periodicals be replaced by full and accurate citations, will have the sympathy of authors, editors and secretaries. This proposal has already been tested in practice. The *British Medical Journal* stopped abbreviating titles in April 1969. A working party set up in 1968 by a Conference of Medical Editors[1] "strongly recommended all journals to follow suit: a majority of (medical) editors agreed to do so". The working party also reported that with full citations "the increase in type area is maximally 2% and usually much less". The editors of the *British Medical Journal* also reported that "the saving in editorial time is enormous".

When the effective use of public money on scientific research is increasingly coming under close scrutiny, any time spent by authors and indeed editors, themselves very often active in research, on such a mundane and unproductive task must be seriously questioned. If any economies result from the present practice, they must only be reflected in the balance sheets of publishing companies.

L. D. Incoll

Department of Plant Sciences,
University of Leeds, UK

Stamps of scientific interest

The British Post Office marked the centenary of the Royal Institute of Chemistry by issuing a set of four multicoloured stamps last month, each featuring a Nobel prize-winning development.

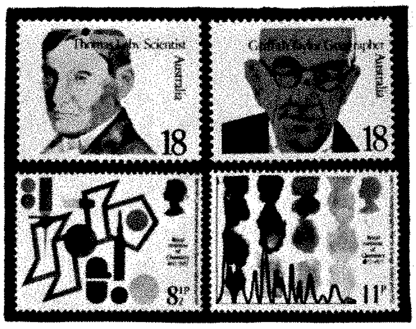
The two shown, at 8½p and 11p, commemorate Professor Sir Derek Barton's work on conformational analysis and the development of partition chromatography by Professor A. J. P. Martin and R. L. M. Synge. They depict the central part of the chemical structure of a steroid against a background of pharmaceutical products and instruments; and a graph, showing the different amounts of substances in a complex mixture separated by partition chromatography superimposed on the kind of pattern produced by the technique when used on paper.

The other two stamps, at 10p and 13p, commemorate the work of Sir Norman Haworth, which resulted in the first synthetic vitamin identical to that occurring in natural citrus fruits, and the work of Professors Sir William and Sir Lawrence Bragg, the father and son who developed crystallography.

Australia last year issued a set of four 18c stamps featuring famous Australian scientists, three of whom ironically were born in England. Sir Thomas Laby (1880–1946) did much of his research on the possible uses of radium in combating cancer. Professor Griffith Taylor (1880–1963) joined the Commonwealth Weather Service in 1910 as a physiographer and then joined Scott's Antarctic expedition.

The other stamps depict the ornithologist John Gould (1804–81), regarded as the father of Australian bird study, and the anthropologist, Sir Baldwin Spencer (1860–1929), who studied the tribal customs of Australian aborigines.

Ian F. Finlay



news and views

Transferable drug resistance in the gonococcus

from J. R. Saunders

GONORRHOEA is currently the most prevalent human communicable bacterial disease. Despite the emergence of strains of the causative organism *Neisseria gonorrhoeae* (the gonococcus) with increasing resistance to penicillin, single high doses of this antibiotic have generally proved effective. The discovery last year of infections in Britain, the Far East and the USA which were refractory to this regimen has caused understandable alarm amongst clinicians (see for example, *Center for Disease Control Morbidity and Mortality Weekly Report* **25**, 261; 1976; Percival *et al.*, *Lancet* **ii**, 1379; 1976; Phillips *Lancet* **ii**, 656; 1976), and the current policy of treating gonorrhoea 'blind' with penicillin has therefore been questioned. The culprits for these recent outbreaks are highly penicillin-resistant gonococci producing the TEM β-lactamase (penicillinase). This enzyme is specified by the widely dispersed transposon A (TnA) and its acquisition by *N. gonorrhoeae* had been widely predicted. TnA is commonly located on R plasmids in the Enterobacteriaceae, *Pseudomonas aeruginosa* and *Haemophilus influenzae* and it seems probable that gonococci have acquired it by contact with one of these organisms.

Almost all gonococci so far studied contain a plasmid of about 2.5 Mdal which has no known function (see for example Mayer *et al. Infect. Immun.* **10**, 1974; Palchaudhuri *et al. Infect Immun.* **11**, 114; 1975). Some gonococcal strains contain an additional plasmid of about 25 Mdal (Stiffler *et al. J. Bact.* **122**, 1293; 1975). But so far all known antibiotic resistances in *N. gonorrhoeae* have been shown to be determined by chromosomal genes. Two groups working independently, Roberts and Falkow (this issue of *Nature*, page 630) and B. I. Eisenstein *et al.* (*Science* **195**, 998; 1977) now show that at least two species of β-lactamase plasmid exist in gonococci, one, from Far East strains, of about 4.4 Mdal, and one, from British strains, of about 3.2 Mdal, in addition to the resident cryptic plasmids. The origin of these resistance plasmids is not yet clear. They may have been received in entirety from other Gram negative bacteria, in particular from *H. influenzae*, which contains similar β-lactamase plasmids (de Graaf *et al. J. Bact.* **126**, 439; 1976). Alternatively they may have arisen by transposition of TnA to resident gonococcal plasmids. The 'donors' of TnA in this case could have been R plasmids which are capable of entering but not subsequently maintaining themselves in *N. gonorrhoeae*. Whatever mechanism is involved, the different plasmid complements of the British and Far East strains suggest that the TEM β-lactamase gene has been acquired by *N. gonorrhoeae* on at least two separate occasions. The 4.4 Mdal β-lactamase plasmid is widely distributed amongst gonococcal strains known to be different on the basis of their nutritional requirements (auxotypes). This implies that considerable interstrain transfer has occurred. It is well known that chromosomal antibiotic resistance markers can be transferred between gonococci by transformation, which is the only mechanism of genetic exchange so far reported in *N. gonorrhoeae*. However, Roberts and Falkow have found that covalently closed circular DNA of either type of β-lactamase plasmid cannot transform *N. gonorrhoeae*. This indicates that transformation plays no significant part in the dissemination between strains of the β-lactamase plasmids.

Roberts and Falkow, and Eisenstein *et al.* have now shown that the 4.4 Mdal β-lactamase plasmid can be transferred by conjugation to other gonococci, to *Neisseria flava* and to *Escherichia coli*. This plasmid is, however, too small to encode sex factor activity itself and only those strains which also carry the 25 Mdal cryptic plasmid can act as donors. The β-lactamase producing strains isolated in the UK, which all lack this larger plasmid, cannot therefore donate penicillin resistance to other bacteria. The 25 Mdal plasmid is thus apparently acting as a gonococcal sex factor and it is of interest that this plasmid has not yet been found in recipient bacteria which have acquired the β-lactamase plasmid from donor strains. This suggests that covalent linkage between the two plasmids is not necessary for transfer but it is not yet clear whether the gonococcal sex factor is totally incapable of transferring itself to other bacteria. However, similar mobilisation of non-self-transmissible plasmids by transfer factors is well documented in the Enterobacteriaceae. It will be of great interest to determine the molecular and genetic properties of this sex factor and whether it is of gonococcal origin.

The discovery of sex factor activity in the gonococcus has serious clinical and epidemiological implications. First, it increases the probability that more strains of *N. gonorrhoeae* will acquire genes for resistance to penicillins and other drugs. It also makes more likely the transfer of resistance genes to a close relative of the gonococcus, *N. meningitidis*, the causative agent of epidemic meningitis. Meningitis is more likely to be fatal than gonorrhoea and the situation is complicated by the fact that *N. meningitidis* is carried without ill effect in the throats of many healthy individuals. Transfer of β-lactamase genes to this organism could occur either directly from *N. gonorrhoeae*, from enteric bacteria, or from *H. influenzae*, also a common inhabitant of human throats and which can cause disease. Furthermore, *H. influenzae* has in recent years acquired both the TEM β-lactamase and the ability to conjugate. In contrast, however, the gonococcal sex factor could benefit research workers by providing a means of genetic transfer in addition to transformation for analysing the genetic determinants of pathogenicity in *N. gonorrhoeae*.

There is obviously a certain irony in the possession of sexuality by *N. gonorrhoeae*. If the gonococcal sex factor determines the synthesis of sex pili, these should provide adsorption sites on donor cells for male-specific bacteriophages. The possession by gonococci of their own venereal pathogens would indeed be divine justice. □

New Uranian satellite belt

from David W. Hughes

On 10 March at about 21.00 universal time the planet Uranus passed across the star SAO 158687 in the constellation of Libra. Reports of this occultation have caused considerable excitement because not only was the star occulted by the planet but it was also occulted by a series of much smaller satellite objects that seem to be in an equatorial belt around the planet.

The occultation was first predicted by Gordon Taylor of the Royal Greenwich Observatory (*J. Br. astr. Assoc.* **83,** 352; 1973). No useful observations of an Uranian occultation had been made before and even for this one the occultation would only be visible from a small area of the Earth. Also Uranus would be about 40 times brighter than SAO 158687 which has a photographic magnitude of +10, so Taylor predicted that the observations would be extremely difficult and would need complex photoelectric equipment.

Occultation observations are important because they can provide data as to the size of the planet, its limb darkening and the extent and composition of its atmosphere. They also provide an accurate spot measurement of the planet's position which is extremely important when it comes to using celestial mechanics to calculate planetary perturbations.

The positions of Uranus, given in the Astronomical Ephemeris, and of the star SAO 158687, given in the Smithsonian catalogue, were carefully checked in January 1977 using photographic plates obtained with the 155-cm astrometric reflector at Flagstaff, Arizona. Franz, Wasserman, Seidelmann, Van Flandern and Marsden (*IAU Circ.* 3038/40) reported that the position of the star had to be corrected by +1.15 arc s in declination. Transit circle observations of Uranus indicated that its declination should also be corrected by −0.22 arc s. This meant that the occultation would not be visible everywhere that Uranus was above the horizon, but would only be seen in an area whose northern limit extended from equatorial Africa, across the Indian Ocean, somewhat north of Perth, Western Australia and then into daylight. There was an uncertainty of about ± 2,500 km in this northern limit caused by the uncertainty in the positions of the planet and star and an additional uncertainty of about 1,500 km due to the fact that the exact radius of Uranus is not known. Occultation would occur near 21 h 00 min UT and the duration would increase as one moved south.

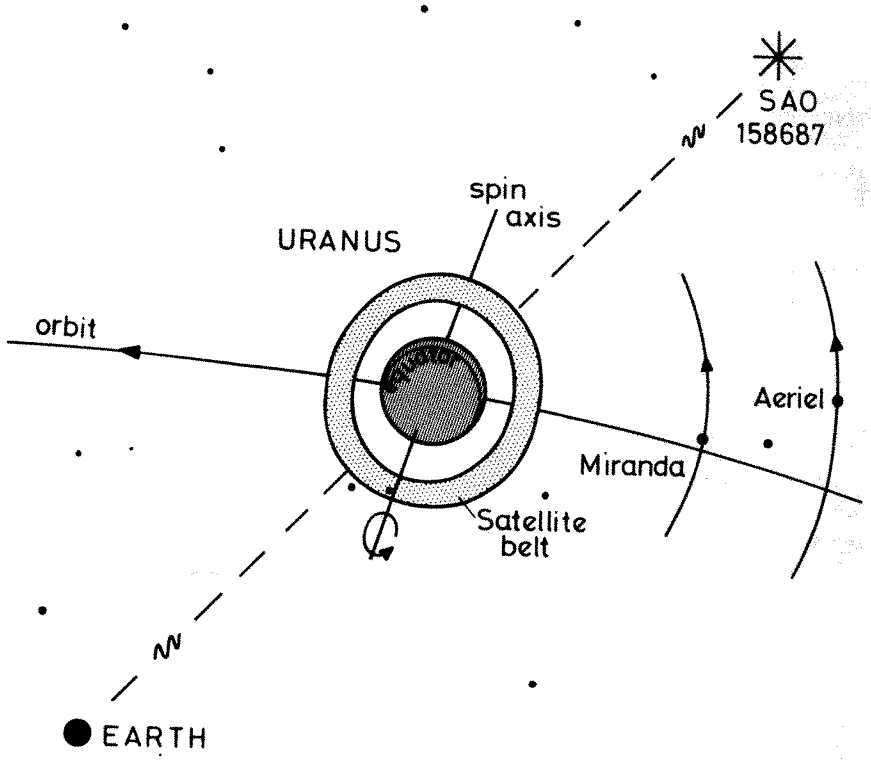


Fig. 1 A schematic diagram of Uranus and its newly discovered satellite belts at mid-occultation on 10 March, 1977. Miranda and Aeriel are the two innermost satellites, SAO 158687, the occulted star, is a 10th magnitude, K5 spectral type star in Libra. The equator of Uranus, the satellite belt and the orbital planes of Aeriel, Umbriel, Titania, Oberon and Miranda are all inclined at about 98° to the ecliptic plane of the Solar System.

On 10 March heavy rain prevented observations in the vicinity of Johannesburg; however astronomers on board the Gerald P. Kuiper Airborne Observatory flying across the southern Indian Ocean about 1,000 miles East of Kerguelen Island (long. 90E, lat. 50S) were more fortunate. The occultation lasted for about 25 min around 21 h 06 min UT. Dunham, Elliot and Mink, the Cornell University astronomers on the plane also, to their surprise, saw a series of secondary occultations that took place during an 8–9 min interval around 20 h 16 min UT and during a similar interval around 21 h 50 min. Bhattacharyya and Kuppuswamy (Indian Institute of Astrophysics) using the 102-cm reflector at Kavalur, Madras saw the secondary occultation start at 20 h 19 min 51 s and last for 8–9 s (they also observed that the star faded by 0.046 magnitude when it went behind the atmosphere of Uranus). Millis, Birch and Trout at the Perth Observatory, Western Australia saw the earlier of the two secondary occultations. All the groups independently concluded that these occultations were caused by satellites that are apparently part of a belt about 48,000 km from the centre of Uranus. This belt is shown schematically in Fig. 1. As Uranus travels along its orbit the left hand portion of the belt occults the star first then the planet occultation occurs. Finally the right hand belt occults.

Brian Marsden (Smithsonian Astrophysical Observatory) has used the times of occultation to compute the following details (*IAU Circ.* 3048, 3051). The belt is circular, in the plane of Uranus' equator, about 12,000 km wide and extends between 42,200 and 54,300 km from the centre of Uranus.

The fact that the times of the individual secondary occultations are remarkably symmetrical with respect to the Uranian occultation suggests that the occulting material is confined to specific narrow rings in the belt. The Kavalur observation seems to have been caused by a 100 km sized satellite near the outer edge of the belt.

Elliot, of the Centre for Radiophysics and Space Research, Cornell University, is continuing the detailed study of the occultation records. It might also be possible to observe the belts directly, although a very large telescope will be required as the brightest satellite is expected to have a magnitude of about 19. At the present opposition of Uranus the belts are between 3.5 and 4.0 arc s to the north and south of the planet and 2.7 to 3.1 arc s to the east and west. ☐

Engineering nitrogen fixation

from Andrew Johnston

> A conference entitled Genetic Engineering for Nitrogen Fixation, organised by Dr A. Hollaender, was held at Brookhaven National Laboratories, Upton, New York, on 13–17 March, 1977.

THE current interest in biological nitrogen fixation reflects the desire that an increased amount of the fixed N in crops should come from this source rather than from energy-expensive fertiliser. The aim of this meeting was to see whether such a goal might be achieved by genetic techniques. Most reports dealt with the status of current research in different aspects of nitrogen fixation, but attention was given to systems whose development might conceivably lead to an extension of the range of nitrogen-fixing organisms.

D. Helinski (University of California, San Diego) reviewed the methodology and uses of DNA cloning. He made the point that to date only the P-group R plasmids can be transferred to many bacteria of agronomic importance. However, their promiscuity and relatively large size make them unsuitable as cloning vehicles and he described methods by which the ability to replicate in a wide range of bacteria might be transferred to more appropriate vehicles.

Detailed mapping of nitrogen fixation (*nif*) genes has been caried out only in *Klebsiella pneumoniae*. C. Kennedy (ARC Unit of Nitrogen Fixation, Sussex) showed by transduction that *nif* genes fall into two closely linked clusters separated by a 'silent' region. Complementation tests have defined a new *nif* gene (*nifL*) which has the attributes of a controlling gene since the mutant is partially dominant and synthesises no nitrogenase components. F. Ausubel (Harvard University) reported that the *nif* cluster proximal to *hisD* could be cloned on the plasmid pMB9.

Turning to the genetics of other nitrogen-fixing bacteria A. Johnston (John Innes Institute, Norwich) demonstrated that the R plasmid R68.45 could promote chromosomal recombination in *Rhizobium* species and that stable recombinants could be formed in crosses between species. A single circular chromosome for *R. leguminosarum* was reported. In *Azotobacter vinelandii* many *nif* mutants have been characterised biochemically, but genetic analysis has been lacking. W. Brill (University of Wisconsin, Madison) reported that *nif* genes in these species could be transferred by transformation, but in contrast to *K. pneumoniae* they were not closely linked. More suprising was the fact that *nif*$^+$ transformants were obtained with DNA obtained from *Rhizobium*.

There have been several reports that plant lectins may determine the specificity of interactions between *Rhizobium* and legumes. D. Bauer (C. Kettering, Yellow Springs) showed that not all strains of *R. japonicum* bind the lectins of its host, the soybean. Those that did not could however, be induced to do so by whole soybean roots. This observation may resolve some of the discrepancies in the literature in which binding of lectins to some infective strains of *Rhizobium* has not been observed.

The symbiosis between *Azolla* and *Anabaena* has been used as a source of fixed nitrogen in Asian rice paddies for centuries. B. Rains (University of California, Davis) demonstrated that an equivalent of 90 kg ha^{-1} yr^{-1} may be contributed by this symbiosis. It is not possible however to cultivate the blue-green alga outside the plant so the goal of breeding for increased levels of fixation remains a distant one.

The role of *Spirillum* as a significant contributor of fixed nitrogen remains enigmatic. Although it was shown by J. Dobereiner (Embrapa, Brazil) to get into maize roots, it appears to contribute little to the nitrogen input of this plant though it may do so with some tropical grasses and wheat varieties.

The supply of energy to nitrogen-fixing systems was considered by several speakers to be a major limitation to increasing levels of fixation. R. Valentine (University of California, Davis) estimated that in the 'artificial nodule' in which NH$_4^+$-excreting strains of *K. pneumoniae* were grown, about 20 molecules of ATP are required for every molecule of nitrogen fixed, although this ratio varied depending on environmental factors. Invariably H$_2$ is liberated during the reduction of N$_2$, but some of this energy loss may be recouped if a hydrogenase is present. H. Evans (Oregon State University, Corvallis) discussed the importance of this reaction in root nodules and pointed out that a particularly effective strain of *R. japonicum* has hydrogenase activity.

If *nif* genes are to be introduced into new hosts suitable vectors must first be obtained. Evidence that DNA from the crown-gall bacterium *Agrobacterium tumefaciens* does in fact integrate into plant DNA was given by E. Nester (University of Washington, Seattle). DNA isolated from crown-gall tissue specifically hybridised with one particular fragment derived from *SmaI* digestion of the large tumour-inducing (Ti) plasmid of *A. tumefaciens*. This integrated plasmid is present in about 20 copies per plant cell and is transcribed in crown gall tissue, since RNA from this source hybridises specifically with it. These results were complemented by those of D. Schell (University of Ghent), who found that in some cases Ti plasmids contain large hairpin loops characteristic of some translocating sequences. In a strain which had lost both the ability to degrade octopene and to induce tumours one of the loops (length 40 μm) was missing.

The use of plant viruses as possible vectors was discussed by R. Meagher (University of Georgia, Athens). He showed that when the DNA of cauliflower mosaic virus was cloned in *E. coli* extra proteins were synthesised, but that none corresponded to normal virus proteins, which confirms the difficulties of obtaining accurate transcription and translation of eukaryote DNA in prokaryotes.

However, it was emphasised at the meeting that the most promising strategy for improving the economic contribution of biological nitrogen fixation to agriculture probably lies in the development of better types of already existing nitrogen fixation systems.

The conference also considered some of the legal, ethical and environmental implications of the scientific contributions. These discussions were strongly influenced by the present concern in the United States about the potential hazards of recombinant DNA research. This meant that while there was a general consensus of opinion that production of more efficient nitrogen-fixing plants and bacteria was desirable, a number of people were concerned about the possible environmental impact of such organisms even if they were formed by classical methods of gene transfer. ☐

Positronium spin conversion

from P. W. Atkins

A TECHNIQUE which can detect minute concentrations of triplet states of molecules in gases at normal pressures may have important applications in detecting small concentrations of some air pollutants. The technique depends on positronium spin conversion, a new development of the well-known interconversion of *ortho* and *para* hydrogen.

The interconversion of *ortho* and *para* hydrogen by paramagnetic species has been known, studied and understood for some time. The effect depends on the two nuclei responding to the magnetic field of the paramagnetic molecule; in a collision one of the protons is closer to the unpaired spin than the other, and so its magnetic moment, and therefore its spin orientation, is changed by a greater amount. This relative reorientation of the two proton spins switches an *ortho* molecule (where the two nuclear spins are parallel) into a *para* molecule (where the nuclear spins are in opposition). The rate of interconversion is not fast because it depends on the interaction of the field of the paramagnetic species with the two nuclear magnetic moments, and these are very small. If the magnetic moments were of the size typical of electron magnetic moments (about 2,000 times larger than nuclear moments), or if the *ortho* and *para* molecules could combine in some way with the radical and by so doing change their spin angular momentum by sharing it with the paramagnetic species, the rate of *ortho–para* conversion would be very much greater.

In a recent letter W. Brandt and D. Spektor (*Phys. Rev. Lett.* **38**, 595; 1977) have, in fact, taken the step which leads to a rapid interconversion rate. They have done this by replacing the hydrogen molecules, with their weak nuclear moments, by positronium, and studying the rate of interconversion of *para* positronium (e$^-$ and e$^+$ spins in opposition) and *ortho* positronium (e$^-$ and e$^+$ spins parallel). The magnetic moments of e$^-$ and e$^+$ are both of the order of a Bohr magneton, and so the (e$^-$, e$^+$) species can act as a sensitive probe for the existence of paramagnetic species in the gas phase (as well as in solids, as had been found previously). Alternatively, the positronium can attach to a paramagnetic species, and by a redistribution of spin angular momentum, switch from *ortho* and *para* while the species switches from triplet to singlet. In these ways positronium interconversion can detect free radicals and, as the authors show, respond to the presence of photo-excited triplet molecules in the gas phase.

The experiment is basically the observation of the quenching of scintillations from positronium in the presence of the impurity of interest (SO_2 or benzaldehyde for example). The positron source was a $5\,\mu$Ci ^{22}Na sample at one end of a 25-cm cylinder of diameter 10 cm, with an ultraviolet source at the other. Two detectors monitored the scintillations; one was placed behind the ^{22}Na source, and the other next to the chamber wall. The standard coincidence counting techniques were used to monitor the delayed coincidences between the 1.28 MeV γ ray from the source (which accompanies positron emission) and one of the 0.511 MeV γ rays from the annihilation of a positron by an electron. The experiment is repeated with and without ultraviolet illumination and coincidences for times greater than about 30 ns were stored. This choice of time delay eliminates positron annihilation from all states other than *ortho* positronium.

The role of the paramagnetic species (such as a photo-excited triplet state of benzaldehyde) is to convert the long-lived *ortho* positronium into *para* positronium, which annihilates rapidly. The authors examined argon, nitrogen, and air at atmospheric pressure with trace impurities, and found that the cross sections for quenching are very large, of the order of 1.5×10^{-12} cm^2 for benzaldehyde and 0.9×10^{-12} cm^2 for SO_2. These values are of the order of $10^4\,\pi a_0^2$, where a_0 is the Bohr radius, and indicate interaction ranges of the order of 50 Å. They appear to increase as $\sqrt{T}$ in the range 300–400 K.

The importance of the effect is its sensitivity, on account of its large cross section, to very small concentrations of photomagnetic trace impurities in air; triplet concentrations as low as 10^{-8} can be detected at atmospheric pressures. The authors speculate that their work will have implications for research on gas pollution, in particular on H_2SO_4 formation by the photochemical oxidation of SO_2 to SO_3 in the atmosphere. □

Molecular human cytogenetics

from Susumu Ohno

> One of the 6th series of annual ICN–UCLA symposia, entitled Molecular Human Cytogenetics, organised by R. S. Sparks and D. Comings, was held at Keystone, Colorado on 6–11 March, 1977.

THE meeting appropriately began with excellent reviews by D. E. Olins (Oak Ridge National Laboratory) on nucleosomes and by E. H. Davidson (CalTech) on DNA sequence organisation. A progress report by Davidson indicated that some of the short interspersed repetitious sequences, each a few hundred nucleotide pairs long, may actually represent the promoter or operator site for an adjacent unique DNA sequence. If RNA polymerase II can be modified by a cell type specific modifier subunit, the polymerase, so modified, can selectively transcribe a specific group of structural genes capped by the similar promoter sites. Thus, this particular class of repetitious sequences is at last gaining functional respectability.

Regardless of whether the functional subunit of nucleosomes is a histone dimer rather than a tetramer (R. Chalkey, University of Iowa), a nucleosome itself unquestionably consists of two histone tetramers that assure the equimolar representation by H2A, H2B, H3 and H4. 160 DNA base pairs may make one or two loops around a nucleosome. The distance between adjacent nucleosomes, however, may be variable. As a rule, it was thought to be represented by 60 exposed base pairs (Olins), but V. Allfrey (Rockefeller University, New York) remarked that in the rat liver, there are 170 exposed DNA base pairs between nucleosomes. In view of a proposed flip flop mechanism involving histone tetramers of neighbouring nucleosomes, this uncertainty about internucleosome distance is annoying to say the least. What was in everybody's mind was the fate of nucleosomes during active transcription and replication of DNA.

With characteristically beautiful photomicrographs, S. L. McKnight (Columbia University, New York), from Oscar Miller's laboratory, seems to have shown that a stretch of DNA, being actively transcribed as evidenced by the presence of RNA polymerase molecules and growing transcripts, seems to be associated with as many nucleosomes as that representing an adjacent spacer sequence. Allfrey, however, was of the opinion that an actively transcribing ribosomal RNA gene of the slime mould, *Physarum polycephalum*, is either not associated with nucleosomes or those nucleosomes that maintain the association are highly modified, by acetylation of histones perhaps. The latter possibility was, of course, not ruled out by McKnight. With a rather indirect but clever method applied to HeLa cells, R. L. Seale (University of Colorada Medical Center, Denver) presented good evidence that, during DNA replication, one-half of a replication fork (therefore two-quarters of a replication loop) still maintains nucleosomes. The apparent omnipresence of nucleosomes forces us to modify much of our thinking as was evident in J. Cleaver's (University of California School of Medicine, San Francisco) discussion of DNA repair enzymes.

From a string of beads, visible under the electron microscope, to a chromosome as seen under the light microscope, needs a great leap of the imagination. H1, which has a hydrophobic globular core and the very basic amino, as well as carboxyl, terminus is a good candidate for maintaining higher order helical packing of the string of beads by bridging nucleosomes at a certain interval. According to D. Cole (University of California, Berkeley) H5 (found in vertebrates with nucleated erythrocytes) as well as some of the so-called HMG proteins of I. Isenberg, can be considered as allies of H1. Furthermore, he has shown that each mammalian species generates not one, but several organ-specific species of H1. Data from sea urchin indicates that the basic histone genetic unit contains one each of the H1, H2A, H2B, H3 and H4 genes. Cole's findings and the similar finding by Isenberg (Oregon State University) for H2A indicates that the genome of higher vertebrates contains not one but several genetic units for histones that are the same with regard to the H4 gene but different with regard to H1 genes and to a lesser extent H2A genes. In this connection, an apparent paradox is noted with great interest. Using H4 message purified from HeLa cells, W. Prensky (Tufts University, Boston) reported that the number of histone gene copies in the human genome is only of the order of tens rather than hundreds and is localised on the short arm of chromosome 7 near its centromere. By contrast not only with sea urchins and frogs but also with the cat, the human genome apparently contains fewer copies of each of the multigene families. This is true for the 5S RNA genes (found on the long arm of chromosome 1) and also of the 18S and 28S RNA genes (distributed among five pairs of acrocentrics—chromosomes 15, 16, 17, 21 and 22).

I wonder if humans owe their extraordinary longevity to the fact that they possess so few copies of these genes. It seems to me that having too many copies of a functionally ubiquitous gene is a curse rather than a blessing to somatic cells. Mutations which alter function (suppressor-type mutations of RNA genes, for example) sustained by a few copies out of hundreds or thousands are not likely to be particularly deleterious, and so may accumulate, resulting in more and more mistranslations and mistranscriptions leading finally to the general deterioration so characteristic of the ageing process. With fewer gene copies, mutated cells will be eliminated more efficiency thus delaying ageing.

Of winged beans and Wom Poms

from Robert M. May

ADMIRERS of Michael Flanders and Donald Swann will recall their hymn to the fabulous Wom Pom plant (Angel Record 35797): "You can do such a lot with a Wom Pom / You can use every part of it too . . . The flesh in the heart of a Wom Pom / Has the flavour of porterhouse steak / And the juice is a liquor / That will get you higher quicker . . . *Gaudeamus* Wom Pom, gladly we salute / *Vade mecum* Wom Pom, philanthropic fruit."

Nature's closest approximation to the Wom Pom may well be the little-known tropical legume, *Psophocarpus tetragonolobus* or winged bean. In its study of underexploited tropical plants with promising economic value (see *Nature* **265**, 209; 1977), a panel of the US National Academy of Sciences under the direction of Noel Vietmeyer decided that the exceptional merits of the winged bean deserved a separate and deeper examination. The fruits of that work are set out in *The Winged Bean: A High-Protein Crop for the Tropics* (NAS, Washington DC, 1975).

Today the winged bean is grown only as a backyard crop in Papua New Guinea and Southeast Asia. These regions are, however, typical of the humid tropical zone that includes large parts of Central and South America, the Caribbean, Africa, Oceania, and West Asia, where protein deficiency is high and the plant is still unknown; not many edible crops with such high protein content will grow in these regions. Unlike the soybean (where only the seeds are used), all parts of the winged bean can be eaten: tubers, seeds, leaves, flowers, and shoots.

The immature pods are highly palatable, and can be eaten raw. The seeds are similar in composition to soybeans, averaging 34% protein (by dry weight) and 17% oil. The seeds give an edible oil, with a good proportion of polyunsaturated essential fatty acids. The tuberous roots are slightly sweet, and contain 20% protein (by dry weight). This is spectacularly higher than the protein content in other edible roots and tubers such as cassava (1%), potatoes (2%), sweet potatoes (2%), or yams (2%).

Like other leguminous plants, the winged bean fixes atmospheric nitrogen by bacteria in nodules on the roots. Compared with other edible legumes, *P. tetragonolobus* appears to have more and larger nodules on its extensive root system, which may account for the plant's exceptional protein content. These factors are also probably responsible for the plant's ability to grow well in tropical soils poor in nitrogen, and in newly cleared soils (without the inoculation with appropriate soil bacteria demanded by many other tropical legumes). It can also be used in crop rotations to restore soil nitrogen.

Little is known about the varieties of *P. tetragonolobus*. Those currently cultivated require staking to produce high yields of pods and seeds. The NAS report acknowledges that this method of agriculture is impractical and uneconomical for mass cropping, and that not all winged bean pods mature at the same time, so that the plant is not likely to rival the soybean as a large-scale commercial product in the near future. "Its potential is as a subsistance crop or as a cash crop for small markets. For these purposes, the fact that it produces food during many months is an advantage, and staking is not a serious handicap".

One of the long term benefits that may well come from research on *in vitro* recombinant DNA is better crops, genetically engineered to our requirements. In the short run, however, we could make better use of what nature has provided, particularly in the tropics.

How a chromosome or rather a chromatid manages to pack 10^8 or more DNA base pairs into itself is a mystery still unresolved. After listening to discussions of sister chromatid exchanges by S. Latt (Children's Hospital Medical Center, Boston) and S. Wolff (University of California Medical Center) and of meiotic segregation by M. Moses (Duke University School of Medicine), one cannot help but marvel at the ease with which two sister DNA strands manage to sort themselves out without too much unwinding. All the tortuous models proposed so far are likely to be well off target. To discuss the mechanism of Q and G-banding and of R-banding without knowing the packing method the chromosome uses is likely to be futile. Although D. Comings (City of Hope Medical Center) pointed out that a 5% difference in GC/AT ratio may make a 50% difference in fluorescence intensity, the fact that anti-single base antibodies (O. J. Miller) as well as G–C specific antibodies (J. H. Van de Sande), can also band chromosomes, seems to indicate that what matters is not the base composition of a whole long stretch but short stretches here and there that occupy exposed or accessible positions on the chromosome. I have amused myself with the thought that a solitary human chromosome left in man–mouse somatic hybrids still exhibits its own characteristic banding pattern, although its chromosomal proteins by then are likely to be entirely of mouse origin.

Because of strenuous afternoon exercise on skis and skates and social evenings not many people attended the last session, on evolution, which I thought the best. Of man's relation to the great apes, both A. Wilson (University of California, Berkeley) and P. Pearson (University of Leiden) pointed out that the genetic distance between the chimpanzee and the gorilla is considerably greater than that between man and the chimpanzee. The tendency of certain autosomes to be extremely conserved was noted both in primates (Pearson) and Equidae (O. A. Ryder). Even rhesus monkeys seem to maintain a homologue of human chromosome 1. Whether such an apparent chromosomal conservation correlates well with the conservation of its individual genes, as is the case with the mammalian X, is an evolutionary question of utmost interest. As no functional significance can be assigned to highly repetitious DNA base sequences (so-called satellite DNA), their evolutionary role cannot be conjectured. Nevertheless, they have a peculiar habit of jumping around, ignoring the pair-by-pair homology of diploid organisms, the human Y-specific repetitious sequences being an exception (L. M. Kunkel, Johns Hopkins Hospital). Human satellite DNAs 1 to 4, some of them more ancient in origin than others, have apparently been jumping around as free spirits during the course of evolution that first branched out gibbons and then the anthropoid apes and man (J. R. Gosden, Western General Hospital, Edinburgh). E. Southern pointed out some years ago that the evolutionary fate of highly repetitious sequences is to become moderately repetitious by accumulation of random mutational base changes. Unless a mechanism exists for eliminating old repetitious sequences, the genome size should continue to increase with every new addition of highly repetitious sequences. While most species have apparently invented this elimination mechanism, the kangaroo rats (*Dipodomys*) of North American deserts have not. Among this group of leaping rodents larger genome sizes are correlated with a greater amount of highly repetitious DNA (F. T. Hatch, Lawrence Livermore Laboratory).

Sex chromosomes continue to unravel their mysteries ahead of autosomes, the *in vitro* study of the X-inactivation mechanism now seems possible. Using three X-linked marker enzymes, G. Martin (National Institutes of Health) reported that mouse ovarian teratoma cells, kept in an undifferentiated state with the help of the feeder layer, express the genetic activities of both X chromosomes. As soon as they are allowed to organise and differentiate one X-chromosome is inactivated. Rapidly accumulating evidence mainly due to S. S. Wachtel has all but verified the proposed testis-organising function of the H-Y antigen (S. Ohno, City of Hope Medical Center). But the actual location of this testis-determining H-Y gene is now problematical. Although the presumptive existence of this gene in multiple copies could still salvage its Y linkage, X linkage of the H-Y gene in the normally inducible state with the Y carrying its activator gene, is becoming increasingly attractive. ☐

Objective authentication

from C. R. Cavonius

Authenticity in Art was the subject of an interdisciplinary symposium at the Van Gogh Museum, Amsterdam, on 12 March 1977. The symposium, which reflected the increasing interest in the use of objective techniques in authentication, was organised by H. L. C. Jaffé (Art Historical Institute, University of Amsterdam) and L. H. van der Tweel (Van Danzig Foundation).

THE discussion clearly showed the complementarity of techniques derived from physical and physiological science, and from modern graphical analysis. S. J. Fleming (Oxford University) described changes in the pigments used by artists over the centuries. The obvious possibility of rejecting a later forgery because it contains pigments that were not available to the original artist has been extended by methods that in some cases enable the dating of different samples of the same pigment on the basis of their radioisotope ratios or trace contaminants. This has proven especially useful in the case of the ubiquitous pigment lead white which, depending on the era in which it was manufactured, contains varying amounts of impurities, or different crystalline structures (although the chemical composition remains the same). Fleming concluded that if museums and collectors regularly used physical dating techniques, the chance of success by forgers would become vanishingly small.

Physical methods are, however, of little help in discriminating between artists of the same school, who tended to use similar materials. This problem was discussed by J. Bruyn (Art Historical Institute, Amsterdam) within the framework of the current exhibition in Utrecht of works by Jan van Scorel. Recent developments in infrared scanning methods make it possible to reveal the artist's sketches under all pigments other than black, and this technique has revealed several cases in which different parts of a painting were sketched by different artists. Bruyn further explained how an analysis of underpainting can aid in authenticating a painting, since members of a given school tend to follow the master's technique, whereas copyists attempt only to reproduce the appearance of the surface.

Prints and drawings present a special problem, since they offer little material for physical analysis. In this case, A. Perrig (University of Hamburg) demonstrated how an analysis of the fine structure of an artist's line can aid in identifying a drawing in cases in which the overall style is ambiguous. As an example, Perrig showed that the cross-hatching in several putative Michelangelos was done with a stroke that does not appear in any of his authenticated works, and through a series of steps that resembled a good detective mystery, traced their origin to Cellini.

Two different methods for describing an artist's style in objective terms were proposed by J. J. Denier van der Gon (University of Utrecht) and J. Storm van Leeuwen (Royal Library, The Hague). The first reported the results of recent experiments in which the velocity and position of an artist's hand were recorded while she sketched several types of line. When drawing repetitive lines for shading, velocity increased in proportion to line length, whereas when copying a line, or drawing an outline, velocity remained essentially constant, suggesting that the two types of activity are mediated by different neuromuscular control systems. If it were possible to deduce from a drawing which types of motion had been used for specific lines, it might be possible to identify copies, since these would presumably consist predominantly of constant-velocity lines. However, Denier van der Gon admitted that this would be technically difficult.

Storm van Leeuwen described his experience with a method that is already available for describing an artist's style in objective terms: the pictological method devised by van Danzig, which he used to conclude that only 33 of 116 paintings that were attributed to Frans Hals in the 1930s were actually from his hand; subsequent authentication through, for example, pigment analysis has in general confirmed van Danzig's analysis. The heart of this method is the identification of at least 100 characteristics in well-authenticated works of a given artist. If a dubious painting contained 75% of these, van Danzig concluded that it was authentic. Storm van Leeuwen reported a high rate of success in applying pictological analysis to objects as diverse as bookbindings, but cautioned that the method is difficult to learn.

In the general discussion the view that connoisseurship must remain the ultimate test of a work of art was put forward. But this traditional view had clearly received a strong challenge from the more objective methods presented. □

Continental drift changes climate

from Peter J. Smith

THE annual mean temperature difference between the equator and the north pole is at present about 41 °C (equatorial 27 °C to polar −14 °C). But evidence from the fossil record makes it clear that temperatures during the Mesozoic, for example, were generally higher than they are now and that the large equator–north pole temperature drop currently observed is probably atypical of the past few hundred million years of geological time. So why has the temperature gradient increased? Why is late Caenozoic climate abnormal? One possibility is that since at least the Palaeozoic the Earth has been influenced by extraterrestrial effects such as variations in solar luminosity, as suggested by Ulrich (*Science* **190**, 619; 1975). However, Donn and Shaw (*Geol. Soc. Amer. Bull.* **88**, 390; 1977) now claim that the appeal to external processes is unnecessary and that climatic changes in the northern hemisphere during the Mesozoic–Caenozoic may be attributed entirely to changing terrestrial geography.

Their evidence comes from the application of a modified form of the thermodynamic meteorological model of Adem (*Tellus* **14**, 102; 1962 and subsequent papers), the purpose of which is to estimate the temperature of the Earth's surface and atmosphere from the radiation balance. The details are complex and can barely be summarised. Suffice it to say that the model involves such factors as the solar radiation received at the Earth's surface, the surface albedo (reflectivity), latent heat loss from the surface by evaporation, heat of condensation in the atmosphere, cloudiness, heat storage in the oceans and atmosphere, the horizontal eddy diffusion of heat, and heat added to the atmosphere by short-wave and long-wave radiation. What emerges from a consideration of these thermal effects is an equilibrium temperature distribution; and the model can certainly be said to work insofar as it has been used successfully to describe the present climate.

The crucial element as far as Donn and Shaw are concerned, however, is the change in the Earth's albedo with time. The present albedo of the Earth as a whole is about 30%; that is, about 30% of the incident solar radiation is reflected back into space and is thus unavailable for warming the Earth's surface and atmosphere. But this average conceals large variations, for whereas the albedo of clouds is about 90%, that of ice and snow is 60–80% and that of certain land and water areas is less than 10%. Moreover, because of its transparency and mobility, water transports heat to a greater depth than does land and is thus more capable of storing heat. The amount of heat absorbed by the Earth's surface, and thus available for later warming of the atmosphere, is therefore dependent upon the relative proportions and positions of the different types of surface cover.

And the proportions and arrangement of continents and oceans in the northern hemisphere have changed considerably over the past 200 Myr because of continental drift and possibly polar wandering. The albedo and heat storage characteristics must likewise have changed. To see if such changes are sufficient to account for the observed climatic changes, Donn and Shaw have therefore used their modified Adem model to determine the surface temperature distribution for the early Triassic (200 Myr ago), the mid-Jurassic (150), the Palaeocene (65), the Oligocene (35) and the present. The point of beginning with the early Triassic is that this epoch preceded the separation of Europe, America and Africa and marked a time when much of the northern hemisphere was water. (Of course, much of the northern hemisphere was also under water during the Cretaceous, when large continental areas were submerged. This period has not been considered in order to avoid having to distinguish between the thermal effects of land and large epeiric seas.)

What the analysis shows is that during the early Triassic the temperature difference between equator and pole over most of the northern hemisphere was only about 20 °C. But as more and more land moved in from the southern hemisphere and drifted towards the north pole over the succeeding 200 Myr, the equator–pole temperature drop rose to about 40 °C, which agrees well with the currently observed value of 41 °C. Throughout this period, however, the tropics remained at 25–30 °C. In other words, the increasing temperature gradient was brought about by a gradual decrease in polar temperature unaccompanied by any significant change in equatorial temperature.

The increasing gradient tied to a nearly constant equatorial temperature inevitably led to a decrease in the overall mean northern hemisphere temperature—in fact, a more or less uniform fall of about 3 °C throughout the 200 Myr. But temperature reductions in the higher latitudes were not only greater but also less uniform. In the 60–70°N band, for example, the annual mean temperature fell by about 5 °C during the first 165 Myr and by about the same over the remaining 35 Myr. During the same intervals the proportion of land in that latitude band rose 0–50% and 50–100% respectively, emphasising again the importance of 'continentality' in producing temperature decline. Incidentally, the model analysis shows that the mean 60–70°N temperature fell below freezing point 10–15 Myr ago, which is in good agreement with the onset of North American glaciation.

In summary, then, it would seem that continental drift is capable of explaining fully the gross changes in northern hemisphere temperature for the past 200 Myr. Whether the same can be said for the southern hemisphere, where the present equator–pole temperature drop is even higher at 77 °C, remains to be seen. Donn and Shaw promise that analysis later. □

100 year ago

BERLIN dealers in delicacies have recently received from the south, and especially from Upper Italy, immense quantities of edible birds which have been captured there in their flight northwards. Unfortunately there were not only snipe, fieldfare, and larks, or so-called "delicacies" among the birds sent, but also singing birds, that are never eaten in Germany, such as goldfinch, thrush, and nightingales. The animals were caught on their migratory flight by means of nets, or surprised during the night and indiscriminately killed. A new indication of the importance of an international law for bird protection!
From *Nature* **15**, 12 April, 522; 1877.

articles

Extended radio galaxies

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Observations of extended radio sources are interpreted in terms of a picture for radio source evolution involving multiple ejections of ram pressure confined plasmons in which instabilities lead to the formation of turbulent hot spots and to in situ *particle acceleration. The hypothesis of ram pressure confined turbulent plasmons leads to a coherent theory of various radio phenomena including compact radio sources, radio tail galaxies and extended double radio sources.*

ALTHOUGH the powerful extended double radio sources were among the first extragalactic radio sources to be extensively studied, a detailed understanding of their nature is still elusive. The ultimate source of the energy liberated in these sources remains a problem, and will not be dealt with here. We present a coherent picture of the observed structural, spectral and polarisation features of the extended double sources, assuming that an adequate amount of energy is released and properly directed in the central region of the parent object.

A natural model for double radio sources assumes the components of a given source to be ram pressure confined plasmons ejected from a galaxy into an external medium. More exotic models have been developed to explain the following difficulties: (1) Rayleigh–Taylor instabilities, induced in the plasmon because of deceleration, may cause disruption of the plasmon[1]. (2) Large adiabatic energy losses are expected because of the expansion of the decelerating plasmon. (3) Recent observations indicate the presence of highly polarised 'hot spots' in some extended sources[2–4]. If hot spots are confined by ram pressure, high densities of thermal matter ($n \sim 10^{-2}$ cm^{-3}) are required to achieve penetration to the observed distance. Polarisation observations, however, indicate that this thermal density is about an order of magnitude smaller ($n \sim 10^{-3}$ cm^{-3}) (refs 2, 3). (4) The relatively high equipartition magnetic field intensity in the hot spots implies synchrotron lifetimes on the order of 10^4 yr (refs 2, 3). This is only of the same order as the light travel time from the central source to the observed distance of the hot spot.

We argue that ram pressure confined plasmons do develop instabilities; however, the spectra and amplitude of the instabilities are severely limited by the bow shock flow. These limited instabilities lead to the formation of hot spots, the development of turbulence and *in situ* particle acceleration, so that the above listed arguments are inapplicable.

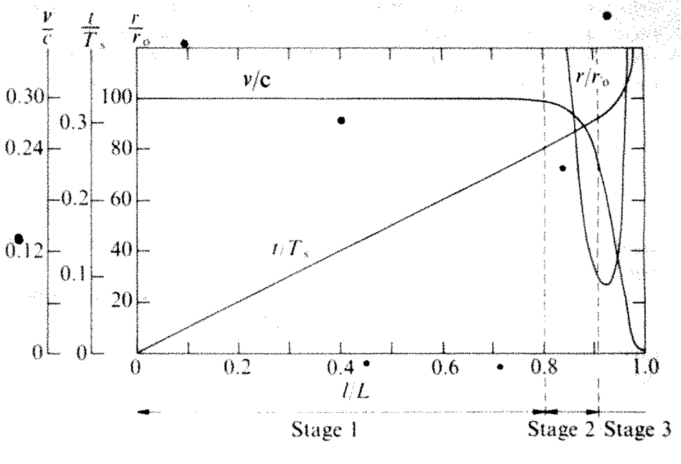
Observations of radio tail objects (and bent double structures) which we assume to be phenomenologically similar to the more energetic doubles discussed here, clearly indicate a number of discrete ejection events (that is, multiple explosions). We therefore adopt a multiple ejection picture (compare ref. 6); this hypothesis (unlike the single ejection) leads to initial nuclear phase luminosities consistent with observations of QSOs.

Plasmons evolution

The evolution of plasmons in a multiple explosion picture for radio galaxies may be separated into four stages (Fig 1).

Stage 1: the plasmon created in the most recent explosion adiabatically expands along the channel formed by the previous explosion[6], resulting in a low surface brightness. Stage 2: this plasmon, on encountering the debris from previous explosions, is rapidly decelerated, adiabatically compressed and greatly brightened. Stage 3: Rayleigh–Taylor instabilities develop near the front of the plasmon, and generate turbulence throughout the plasmon, leading to the acceleration of particles by the betatron and second order Fermi mechanisms[7,8]. Stage 4: deceleration drops to zero and the plasmon expands.

Fig. 1 Velocity of the front of the plasmon in units of light velocity (V), travel time in units of stopping time t/T_S, and a scale height r/r_0 ($r_0 = 1$ kpc) of a plasmon in a channel as functions of the position of the front of the plasmon relative to the length of the Channel l/L. Channel length: diameter is 10. The mass of the plasmon is 0.1 of the total mass of the debris at the end of the tunnel from previous events.



The instabilities no longer grow and the turbulence (and consequent particle acceleration) decays.

Instabilities and hot spots

Linearised calculations indicate that ram pressure confined plasmons are Rayleigh–Taylor and Kelvin–Helmholtz unstable. It seems that the instabilities grow rapidly enough to disrupt the plasmons[1].

Near the front of the plasmon the Rayleigh–Taylor instability is dominant with a growth time $\tau \sim (\lambda/|A|)^{1/2}$, where λ is the instability wavelength and $|A|$ is the absolute value of the plasmon acceleration. For ram pressure confined plasmons $|A| \sim \rho_B v^2/\rho r$, where ρ_B is the background gas density and v, ρ and r are the centre of mass velocity, average density and scale height of the plasmon, respectively. Therefore

$$\tau \sim (\rho/\rho_B)^{1/2} (\lambda r)^{1/2} v^{-1} \qquad (1)$$

As $|A|$ is largest at the beginning of Stage 3 (Fig. 1) the growth rate of all instabilities should be most rapid at this time, and the existence of hot spots and bright ridges, which we associate with growing instabilities, should be most prominently associated with this stage.

Previous calculations did not specifically take into account the interaction between growing surface instabilities and the two-dimensional hypersonic flow of shocked ambient gas around the ram pressure confined plasmon. This flow does not stabilise the surface in the sense that it prevents the growth of instabilities but it does strongly limit the amplitude of the instabilities by sweeping them around the nose of the plasmon into its wake. Therefore, these instabilities contribute to turbulence but are swept away before they reach amplitudes large enough to disrupt the plasmon.

At the stagnation point, the flow velocity of the shocked gas is zero. Above the stagnation point, however, the shocked gas flow has a non-negligible component normal to the surface[10]. Instabilities growing near the stagnation point encounter this flow as soon as they protrude above the surface and are efficiently suppressed.

Away from the stagnation point, the flow is nearly tangential to the surface. The tangential acceleration, A_T, of an instability due to the ram pressure of this flow is thus

$$A_T = (k\rho_{sh}v^2_{sh})/\rho\lambda \qquad (2)$$

where k is a dimensionless constant of order unity, ρ_{sh} and v_{sh} are the density and tangential velocity of the shocked gas, respectively. Numerical calculations[10] indicate that

$$\rho_{sh}v_{sh} \sim \rho_B v$$

$$\text{and} \qquad v_{sh}/v \sim \sin\alpha + 2.2(h/r)\cos^3\alpha \; (h<0.1r) \qquad (3)$$

where α is the angle between the plasmon's velocity and the surface normal, and h is the normal distance above the surface. Instabilities continue to grow until the velocity of the instability relative to the plasmon exceeds the signal speed in the plasmon, v_s. The instability then loses communication with the rest of the plasmon, is decoupled, and ceases its growth. For ram confined plasmons, $v_s \sim v\,(\rho_B/\rho)^{1/2}$, and the time required for decoupling an instability is

$$t_{dc} \sim v_s/A_T = (\rho_B/\rho)^{1/2} v A_T^{-1} \qquad (4)$$

Well away from the stagnation point ($\cos^3\alpha \ll \sin\alpha \sim 1$), $A_T \sim \rho_B v^2/\rho\lambda$. Therefore, $t_{dc} \sim (\lambda^2\rho/v^2\rho_B)^{1/2}$, and one may conclude that $t_{dc} < \tau$ for any $\lambda < r$. This means that Rayleigh–Taylor instabilities do not develop far from the front of a plasmon, and is consistent with observations which seem to indicate that bright hot spots (which we identify with large amplitude instabilities) are always found near the leading edges of extended sources.

Near the front of the plasmon ($\cos^3\alpha > \sin\alpha \sim 0$), the tangential acceleration of an instability depends on the value of h (which is equal to its amplitude). During the linear phase of instability growth

$$t_{dc} \sim (\rho/\rho_B)^{1/2} (\lambda r/hv) \qquad (5)$$

where h is the average amplitude of an instability in the interval before it is decoupled. Comparing this result with the growth time (equation (1)) we conclude that instabilities near the front of the plasmon for which $(\lambda r)^{1/2} < h$ would not develop large enhancements in their amplitudes before being swept away. A natural upper limit on h is the thickness of the shocked region, $h < 0.1r$. Therefore, although the initial spectrum of instabilities is unknown, it is clear that any instability for which $(\lambda r)^{1/2} < 0.1r$ will not develop a large increase in its amplitude.

In conclusion, only a limited spectrum of Rayleigh–Taylor instabilities having wavelengths in the range

$$0.01r < \lambda < r \qquad (6)$$

are capable of developing, near the front of a plasmon, the large increases in amplitude which are necessary for the enhancement of magnetic field and brightness observed in hot spots. Indeed, the sizes of observed hot spots fall in this range[2–4]. Furthermore, there is no evidence for structure in extended radio sources at a scale smaller than the preceding lower limit[4].

Later in Stage 3, $\rho_B/\rho \to 1.0$ and $|A| \to 0$. As a result, the instabilities stop growing, expand and blend with the extended source.

Acceleration and spectra

Equipartition magnetic fields in double-lobed sources (especially in the hot spots) lead to unacceptably large synchrotron losses in times short compared with the lifetime of the radio component. In particular, the frequency, ν_T, at which synchrotron losses become dominant (the frequency where the spectrum should begin to exhibit exponential steepening) is predicted to be within or possibly below the radio window.

This is not the case, however, for our model, in which the position of ν_T in the source's spectrum does not move steadily to lower frequencies. Because of the compression, betatron and Fermi accelerations which occur at various stages of the plasmon's evolution, ν_T at times increases.

The general form of the function $\nu_T(t)$ is[11]

$$\nu_T = [(\text{const.})H(t)\exp(2a)]/[(\textstyle\int H^2(t)\exp(a)\mathrm{d}t)^2] \qquad (7)$$

where a is the time-dependent sum of the coefficients of particle energy change (for example, Fermi acceleration coefficient, adiabatic loss coefficient, etc.). (See Pacholczyk and Scott[7,8].)

During Stage 1, equation (7) implies that ν_T decreases, although synchrotron losses are minimal, since the plasmon is expanded to a large scale size and the magnetic field is small.

The compression during Stage 2 implies that ν_T increases as r decreases

$$\nu_T \simeq 8.2\times10^{23}\, H^{-3}(r_2)(\langle\dot{r}\rangle^2/r_2^2)(\ln r_2/r_1)^{-2}\ (\text{Hz}) \qquad (8)$$

where subscripts refer to the ends of respective stages. The development of turbulence during the early parts of Stage 3 leads to a further increase in the magnetic field through the turbulent dynamo effect, implying that although r increases during Stage 3, ν_T also increases (compare equation (7)). A given component will not be perfectly homogeneous since turbulence develops at different rates in different parts of the source. This means that the frequency of the exponential cutoff, ν_T, will be a function of position in the source, with the regions where the instabilities are most highly developed (that is, hot spots) having the largest value of ν_T.

The overall spectrum early in Stage 3 represents the composite of a series of different exponential cutoffs corresponding to different parts of the plasmon. This implies that the total spectrum will begin to curve (but not exponentially cutoff) at a value of ν_T corresponding to parts of the plasmon where turbulence has not significantly developed. This value of ν_T

is that at the end of Stage 2 (compare equation (8)), which is the point in the evolutionary sequence just before the onset of turbulence. For the observed scale size (6×10^{22} cm), equipartition magnetic field (6×10^{-5} G), and $\langle \dot{r} \rangle \simeq$: $0.1c$ appropriate to Cyg A, we predict that the spectrum should start to bend at

$$\nu_{\text{bend}} \simeq 10^{9}\ \text{Hz} \tag{9}$$

a value close to that observed in this source[6], although uncertainty in this value is relatively large.

As turbulence develops first at leading edges of plasmons, high resolution observations of sources in the early parts of Stage 3 should show less curvature (higher average ν_T) and flatter spectra at the front of the components than at the rear. There are some observational indications that this is so in Cyg A (ref. 2).

Sources well into Stage 3, with fully developed turbulence throughout the entire plasmon, will have lower overall polarisation (because of the turbulence), and ν_T will be shifted to higher frequencies through the source (see equations (7)–(9)) than younger, less polarised sources. In addition, because the plasmon is expanding during Stage 3 the integrated luminosity gradually decreases although acceleration is moving ν_T to higher frequencies so that for a given event the maximum luminosity is reached early in Stage 3.

Depolarisation and energetics

For Cyg A the diameter of the hot spots is ~ 3 kpc. The polarisation observations[2,12] imply a cellular structure with $N = 10$ cells along the line of sight through the hot spot[2] and a value of $nH \sim 5\times 10^{-7}$ G cm^{-3}, where n and H are the thermal particle density and magnetic field in the hot spot. Assuming, since the hot spot is compressed, $H = \eta H_{\text{EQ}}$ where H_{EQ} is the equipartition field for the entire plasmon, the value of the compression coefficient, η, can be determined by comparing the surface brightness of the hot spot with the total surface brightness of the plasmon

$$\eta^{\alpha+1} = \left(\frac{F_{\text{HS}}}{F_{\text{total}}}\right)\left(\frac{r_{\text{total}}}{r_{\text{HS}}}\right)^{2}$$

where $F_{\text{HS(total)}}$ and $r_{\text{HS(total)}}$ indicate the observed flux and radius of the hot spot (entire plasmon), respectively, assuming $F(\nu) \sim \nu^{-\alpha}$. For Cyg A, $\eta = 3.6$; therefore $n = 2.3\times 10^{-3}\text{cm}^{-3}$. The identification of hot spots as instabilities invalidates the application of the ram confinement condition to the hot spots[12] themselves. This condition (properly applied to the entire plasmon) is consistent with the density derived above. Given the velocity, $v \sim 0.1c$, and the density derived from polarisation arguments above, the total kinetic energy of the plasmon is $E_{\text{KE}} \sim 2\times 10^{61}$ erg. The ratio of the particle and field equipartition energy to the kinetic energy is then $E_{\text{EQ}}/E_{\text{KE}} = 1.3\times 10^{-2}$. Therefore about 1% of the bulk kinetic energy of the plasmon has been converted through instabilities and turbulence into relativistic particle and field energy. The value for this ratio is consistent with the value of 2% derived previously in the plasmon model for the head–tail radio sources[7].

Faraday rotation

The multiple explosion hypothesis predicts the existence of non-luminous relic plasmons outside the currently active radio-emitting sources. There is evidence that high-density relics may exist near Cyg A. Observations indicate that the south-following (Sf) component has a rotation measure of -1100 rad m^{-2} while the north preceding (Np) has a rotation measure of $+215$ rad m^{-1} (refs 2, 13). This large difference in the rotation measure between the two components can be interpreted as a result of the source being viewed along a line of sight which intersects one of the relic plasmons. If the source axis is not perpendicular to the line of sight (Fig. 2), radiation from the Sf component passes through a relic plasmon before reaching the observer while radiation from the Np component does not. The difference in the rotation measures would then be a measure of the magnetic field intensity (H_{relic}), thermal particle density (n_{relic},) and diameter (d_{relic}) of the relic plasmon, that is, $H_{\text{relic}} n_{\text{relic}} d_{\text{relic}} N^{-1/2} = 10^{15}\ \Delta RM$, where $\Delta RM = 1315$ rad^{-2}, and N is the number of magnetic cells along the line of sight through the relic.

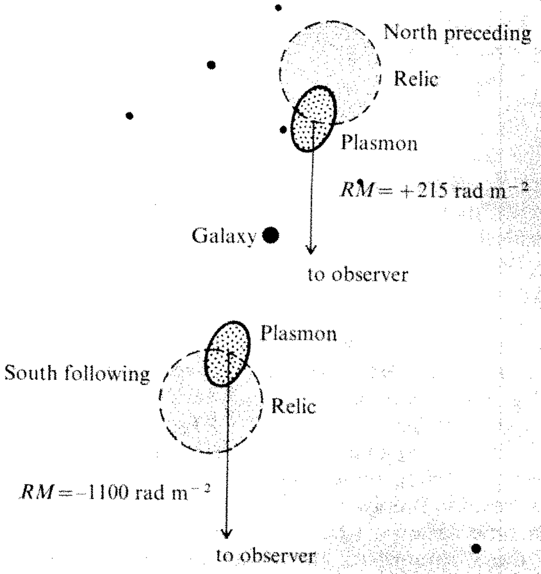


Fig. 2 Schematic representation of the radiating plasmons and relics in Cygnus A. The observed large rotation measure of the south-following component is understood in terms of the large path length of the radiation through the relic plasmon.

The largest differences in rotation measure should occur for sources with high-density relics when the most recent plasmons are in Stage 2 or early Stage 3 and the radio axis lies near the line of sight. Later in Stage 3 when coalescence of the most recent plasmons with relics is nearly complete the rotation measure differences will disappear.

Also, for Cyg A the Sf component must be closer to the observer (Fig. 2) Therefore, because of the difference in light travel time for the emission from the two components, the Sf component should be more advanced in its evolution. If, as was assumed earlier, Cyg A is located at the beginning of Stage 3 in its evolution, the fact that the Sf component is slightly older should imply: (1) Sf luminosity > Np luminosity; (2) Sf polarisation < Np polarisation; and (3) the hot spot in Sf should have a higher surface brightness. All three effects are observed.

X-ray emission

Several authors have pointed out that the X-ray emission from the bow shocks of ram pressure confined plasmons will be relatively small. For Cyg A the predicted bow shock X-ray luminosity would be $\sim 10^{42}$ erg s^{-1} (ref. 14). In addition, the inverse Compton X-ray emission from the interiors of active radio sources is not large[15]. The detection of an X-ray source associated with Cyg A with a luminosity of 2×10^{45} erg s^{-1}, was reported, however, and thermal bremsstrahlung from an extended region of high temperature gas ($T \gtrsim 10^{8}$ K) was suggested[14].

We attribute this X-ray emission to a thermally relaxed ($T \sim 5\times 10^{8}$ K) relic plasmon whose density and mass may then be estimated

$$n_{\text{relic}} 0.51\ d_{\text{relic}}{}^{-3/2}\ (\text{cm}^{-3})$$

$$M_{\text{relic}} \lesssim 1.15\times 10^{43}\ d_{\text{relic}}{}^{32}\ (\text{g}) \tag{10}$$

where d_{relic} is the diameter of the relic cloud in units of 10 kpc. Using density and rotation measure, we can estimate the relic magnetic field

$$H_{\text{relic}} \gtrsim 3\times 10^{-8}\ d_{\text{relic}}{}^{1/2}(\text{G})$$

Assuming $n = 2\times 10^{-3}$ (see preceding section) the mass of the currently active source in Cyg A is $M_{\text{act}} \sim 2\times 10^{42}$ g.

Although the observed X-ray source undoubtedly is partially composed of heated intergalactic gas, a reasonable estimate of its mass would be

$$M_{\rm relic} \sim M_{\rm act} N_{\rm inj} \qquad (11)$$

where $N_{\rm inj}$ is the number of plasmons accumulated in the relic over the lifetime of the source. An upper limit on the number of explosions is

$$N_{\rm inj} \lesssim 7\, d_{\rm relic}{}^{3/2} \qquad (12)$$

The observed X-ray luminosity can be maintained by the injection of an energy equivalent to the currently active source in Cyg A at a rate of ~ 1 (10^8 yr^{-1}). This implies $N_{\rm inj} \lesssim 100$, so $d_{\rm relic} \gtrsim 60$ kpc, $H_{\rm relic} \gtrsim 7 \times 10^{-8}$ G, and $M_{\rm relic} \lesssim 10^{11} M_\odot$.

It is interesting that with these relic parameters, the fastest process for filling the channel through which new plasmons must travel is simply back filling by the relic gas. This occurs on a time scale ~ (the channel length)/(relic sound speed). For these parameters, the filling time is $\sim 10^8$ yr, a value consistent with $N_{\rm inj} \approx 100$, and for which the channel will remain continuously open.

Morphology

The model of radio sources presented here emphasises (1) the multiple ejection of plasmons, and (2) the *in situ* acceleration which occurs when these plasmons are significantly decelerated by ram pressure. The predicted morphology resulting from these effects should be consistent with observations.

Sources with prominent hot spots should be associated with the early parts of Stage 3. Examples other than Cyg A are 3C 33, 3C 390.3 and 3C 61.1. (The latter source also shows a strong 'bridge' feature which can be ascribed to the reflection wave generated as the plasmon 'bounces' off the relic at the beginning of Stage 3).

Other possibilities include subsequent events which occur quickly enough so that the expanded relic is still visible when the latest plasmon encounters it. An example of such a source might be DA240.

If the channel is not straight (because anisotropic ram pressure occurs when there is a density gradient in the external medium[17,18]), some deceleration of the plasmons will occur soon after they leave the parent Galaxy, as the plasmons hit the curved wall of the channel. This causes brightening of the component close to the galaxy, resulting in such structures as bent doubles and wide-angle tails (for example, 3C465, 3C338, 1231+674, 1433+553) (ref. 19). Such structures are found in clusters of galaxies where density gradients are likely to be found.

If the velocity of ejection of the plasmons is comparable with the speed of motion of the Galaxy through the ram-confining medium, subsequent plasmons are not ejected into the channel of the previous plasmoid. Each ejected plasmon is then decelerated by the external medium, and the resulting structure is a radio tail.

In both bent doubles and tails the deceleration process is more gradual than it would be in the case where the plasmoids encounter a massive relic. This implies that the brightness for these two cases should be smaller than for straight doubles. This effect seems to be borne out by observations[19].

Because of the density gradients likely to be present, and the acceleration of the parent Galaxy, we expect straight channels to be rare in clusters of galaxies, and bright straight doubles to be rare in clusters. The exception would be cD galaxies which should lie at the centre of the density gradient and should not be significantly accelerated. Indeed, it seems bright straight doubles are rare in clusters, the exceptions being large centrally located cD galaxies.

Summary

We suggest that components of extended radio galaxies are merely plasmons, and that multiple ejections of these objects lead naturally to the observed structures of double-natured radio sources. The ram pressure deceleration of these objects (which for objects like Cyg A occurs at the end of a channel 'ploughed' by a previously ejected plasmon) leads to instabilities in the plasmon, which, far from destroying the plasmon, lead to *in situ* betatron and Fermi acceleration of the relativistic particles present in the plasmon. This acceleration provides a means of transforming a fraction of the tremendous bulk kinetic energy of the ejected plasmon into relativistic particle (and magnetic field) energy, thus producing the observed radio brightness. This replenishment of internal energy is necessary (and possibly sufficient) to explain the observed spectral and morphological features of extended radio sources.

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Was there a late-Würm Arctic Ice Sheet?

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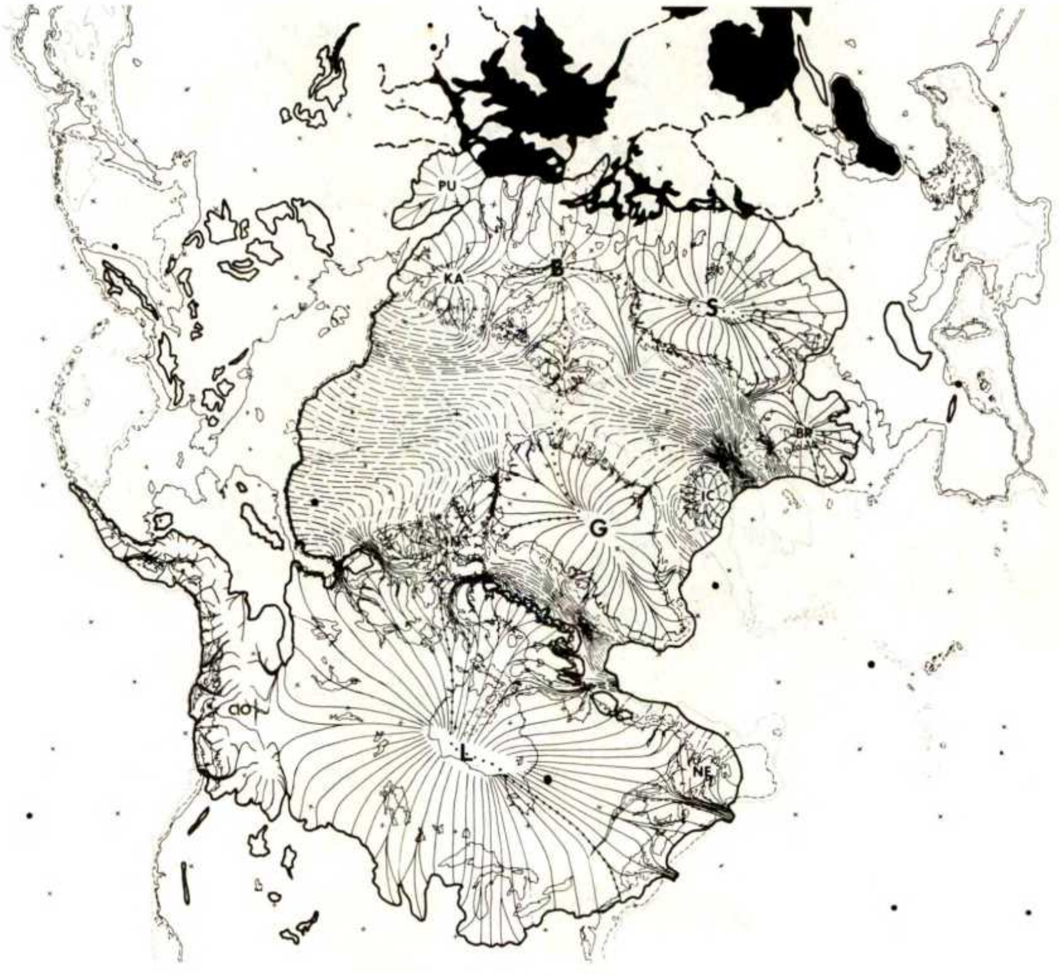
A simplification and synthesis of late-Würm Northern Hemisphere glaciation is possible by postulating an Arctic Ice Sheet that behaved as a single dynamic system.

LATE-WÜRM Arctic glaciation is better understood if we first identify the important features of present Antarctic glaciation. The Antarctic Ice Sheet is a mass of ice and snow covering most of the Antarctic continent, with both grounded and floating portions that are thick enough to flow under their own weight. Ice domes are centres of spreading in the grounded portions and are connected by ice divides. Terrestrial domes are grounded largely above sea level on the East Antarctic continental shield and marine domes are grounded largely below sea level on the West Antarctic continental shelf. Seaward flow from ice domes commonly converges on fast-moving currents of ice called ice streams. Ice streams draining terrestrial ice domes begin as outlet glaciers in fjords eroded through coastal mountains. Ice streams draining marine ice domes begin in submarine troughs eroded on the continental shelf. Ice streams are centres of spreading in the floating portion of an ice sheet, and form floating ice tongues that can coalesce with each other or with thick sea ice to create ice shelves; these are generally confined in embayments and pinned by islands or submarine rises. Calving bays form along the deep-water margin of the ice sheet where icebergs continuously calve from ice that is afloat or grounded well below sea level. Catastrophic retreat of a calving bay up an ice-stream occurs when the floating portion becomes unpinned and the grounded portion surges. A surge may be temporary, periodic or continuous, and is a sudden acceleration of glacial flow caused by substantial ice-bed uncoupling, resulting in massive fracture of the ice surface.

Conventional reconstructions[1] of late-Würm glaciation emphasise the terrestrial character of the Laurentide and Scandinavian ice domes, but when isostatic depression is considered, large portions of these domes were marine, as were the Innuitian, Barents Sea, Kara Sea and East Siberian Sea domes. We have now reconstructed all of the late-Würm ice domes (CLIMAP 18,000 BP revised ice-sheet reconstructions, unpublished data, University of Maine) and find that Weertman's[2] idealised two-dimensional analysis of an ice dome–ice shelf junction predicts that late-Würm marine domes may have been inherently unstable for present combinations of bed slope and sea level along flowlines of major ice streams that drained these ice domes. A conclusive demonstration of whether such instability existed would require a three-dimensional analysis that considers constraints imposed on ice streams by bedrock walls and sills, and constraints imposed on ice shelves by confining embayments and pinning points[2–5]. A preliminary analysis[6] indicates, however, that viscoplastic instability restricts the side-wall constraint on ice streams to a zone only a few kilometres wide, so that the major late-Würm ice streams should conform reasonably well to Weertman's two-dimensional

Fig. 1 A minimum reconstruction of the late-Würm Arctic Ice Sheet, in which an ice shelf covering the Arctic Ocean flowed from the Canadian-European sector to the Alaskan–Siberian sector, where it grounded along the continental-shelf margin and ablated slowly by melting. We assume that marine portions formed mainly from transgression of land portions on to continental shelves exposed by falling sea level. Present sea-level contour and present lacustrine shorelines — — —, Present 100-m bathymetric contour and a conservative late-Würm sea level contour . . . . . , Present 1000-m bathymetric contour and, except for island arcs and ocean ridges, the approximate continental-shelf margin, ———, Limit of late-Würm glaciation. ——— — —, Surface flowlines on the respective grounded and floating portions of the late-Würm Arctic Ice Sheet. L, Laurentide; G, Greenland; B, Barents; S, Scandinavian major domes of the Arctic Ice Sheet. IN, Innuitian; CO, Cordilleran; NE, Newfoundland; IC, Iceland; BR, British; PU, Putorana; KA, Kara; and (in Fig. 2) ES, East Siberian minor domes of the Arctic Ice Sheet. . . . . . , portion of the Arctic Ice Sheet that drained into the Atlantic Ocean. Regions flooded by ice-dammed rivers (black areas) include Mansi Lake in the Ob River basin, the Aral-Caspian Sea, and the Black Sea, which were 128 m above, 48 m above, and at least 50 m below present sea level, respectively, and were interconnected. +, Intersections of 10° longitude and latitude intervals.

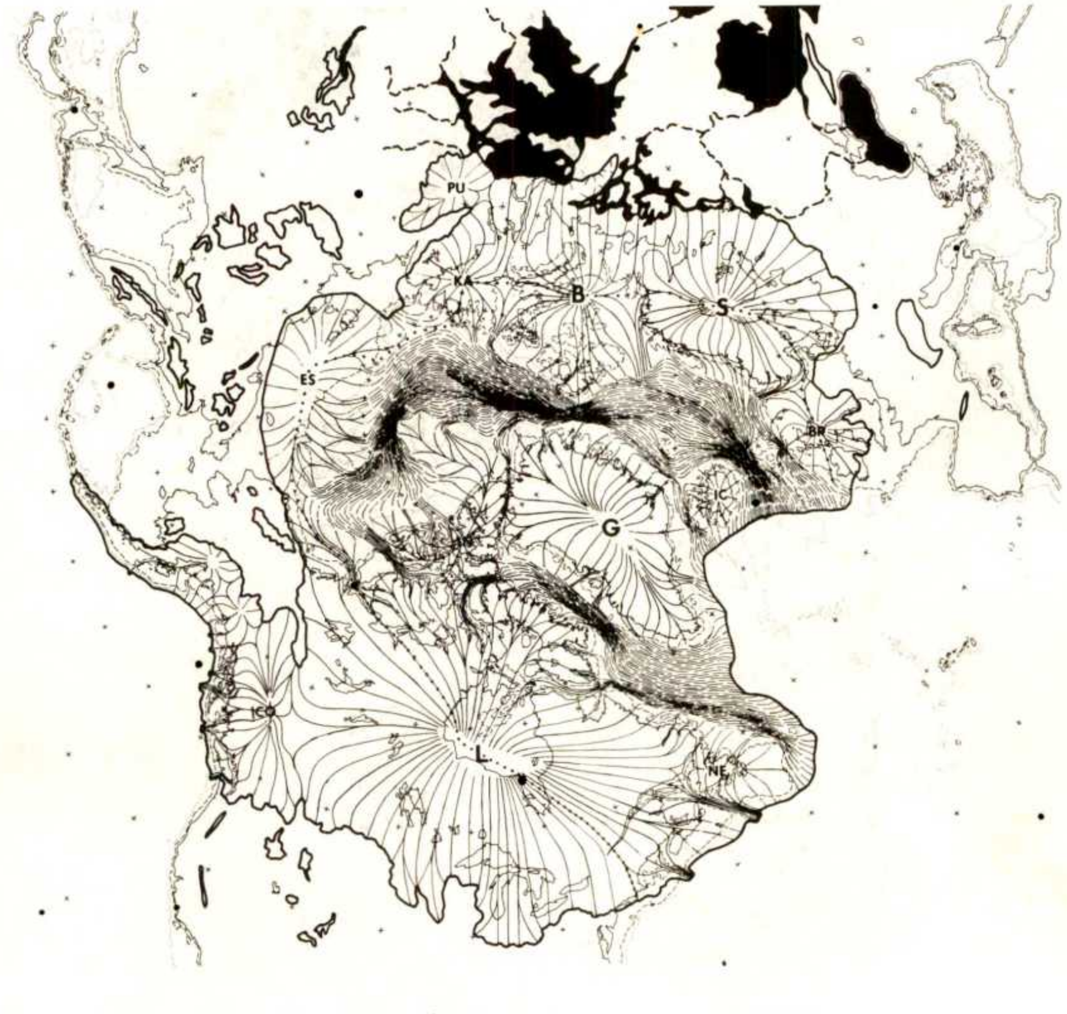


analysis. If so, the major constraint preventing disintegration of inherently unstable late-Würm marine ice domes was probably the inertia of confined and pinned ice shelves buttressing ice streams that drained marine ice domes. This hypothesis is supported by investigations of late-Würm recession of marine West Antarctic ice from the edge of the Antarctic continental shelf[2–9]. Today, this dome, which occupies less than half of its late-Würm area, is drained almost entirely by large ice streams that are usually buttressed by confined and pinned ice shelves. A dynamic argument can be made that removal of the ice shelves would lead to ice-stream surges[2–6], drawing down the ice dome and perhaps leading to its collapse by propagation of calving bays along evacuated ice-stream channels. Therefore, we conclude that reconstructions of late-Würm glaciers may have to consider the inertial constraint of confined and pinned ice shelves on ice streams that drained marine ice domes.

A second difficulty of standard reconstructions involves the chronology of late-Würm deglaciation. Available ^{14}C dates indicate major retreat of peripheral terrestrial ice early in the deglacial hemicycle as opposed to relatively late major recession of northerly marine ice. For example, considerable fluctuating recession began in the southern and western terrestrial Laurentide ice shortly after 18,000 yr ago, whereas major recession of northern Laurentide and Innuitian marine ice apparently did not begin until considerably later in the deglacial hemicycle. The reason for this is not obvious, particularly because the northern marine ice probably was unstable and because a glacial record from northern Alaska[10] suggests that late-Würm climatic amelioration was nearly synchronous in this portion of the Arctic and in temperate latitudes.

In view of these problems in dynamics and deglacial chronology, we think that a simplification and synthesis of late-Würm glaciation is possible by postulating an Arctic Ice Sheet that behaved as a single dynamic system composed of terrestrial and marine grounded ice, and ice shelves (Figs 1 and 2). We start by identifying numerous ice streams in marine portions of the Arctic Ice Sheet from geological literature and from continental-shelf topography. In order to buttress the largest ice streams, we endorse the concept of an ice shelf on the Arctic Ocean[11–13] and postulate extensions of this ice shelf across the Greenland, Norwegian and Labrador seas into the North Atlantic. Ice-shelf flow was determined by junctions of ice shelves with domes of the Arctic Ice Sheet. Two Arctic Ice Sheet reconstructions are presented because the distribution of late-Würm Arctic ice is not well known. In both the minimum (Fig. 1) and maximum (Fig. 2) reconstructions, the Greenland and Norwegian seas

Fig. 2 A maximum reconstruction of the late-Würm Arctic Ice Sheet, assuming marine portions formed mainly from thickening sea ice which became grounded on continental shelves. Markings are described in Fig. 1 legend. Compared with the minimum reconstruction in Fig. 1, the maximum reconstruction in Fig. 2 shows a hypothetical ice dome in the East Siberian Sea as an integral part of the Arctic Ice Sheet, an expanded Innuitian ice dome, and a more southerly position of the ice-shelf calving margin in the North Atlantic. These features greatly increased the area of the Arctic Ice Sheet that drained into the Atlantic Ocean. Circumstantial evidence for the hypothetical ice dome in the East Siberian Sea included the glaciated character of the De Long Islands, the presence of foreign till and erratics on the north coast of Alaska[14,15]; the truncated and dissected top of the Chukchi Cap[16]; and the topography of the sea floor in the area of Chukchi Cap, which appears glaciated and is very similar to sub-ice topography in East Antarctica (compare bathymetric contours of the Map of the Arctic Region, world 1 : 5,000,000 sheet 14, American Geographical Society, New York, 1975, with topographic contours under the East Antarctic Ice Sheet[17]).



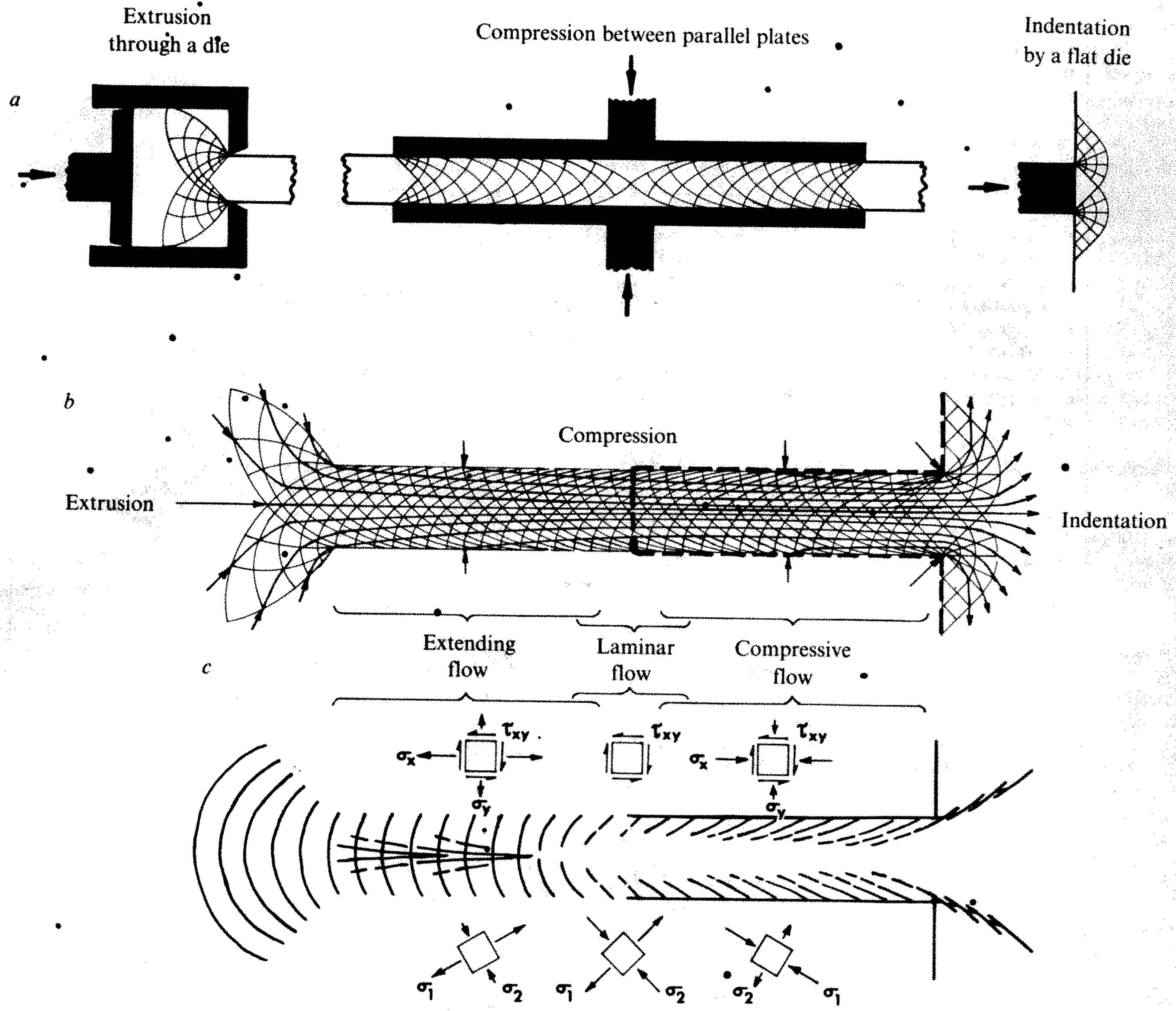


Fig. 3 The dynamics of ice streams draining marine portions of the Arctic Ice Sheet. The orthogonal slip-line fields of maximum shear stress for extrusion, compression and identation (*a*) can be joined to give the approximate slip-line field and flow lines for an ice stream (*b*) and to predict the expected crevasse pattern (*c*) when an ice stream is the major conduit for flow from an ice dome to an ice shelf. The channel eroded by an ice stream allows the ice dome–ice shelf junction (— — —) to extend part way up the ice stream. Flowlines (→) converge on fjords through the partly subglacial mountains bordering the continental shield at the head of an ice stream, and diverge on to the ice shelf bordering the continental shelf at the foot of an ice stream. Surface components of the stress tensor σ_{ij} and its principal stress components σ_k, shown on opposite sides of the ice stream, relate crevasses to extending, laminar and compressive flow as the ice stream becomes ungrounded and merges with the ice shelf. Removal of the ice shelf would bring the extending-flow regime to the foot of the ice stream, allowing a calving bay to migrate up the ice stream as icebergs are sectioned along extending-flow crevasses. Calving-bay migration would stop when the ice-stream grounding line reached a bedrock sill above sea level at the head of the fjord through the mountains, or would proceed into the heart of a marine ice dome if a sill did not exist.

were covered by a southward-moving ice shelf that was nearly grounded on the shallow banks between Greenland, Iceland, the Faeroe Islands and Scotland; and was fed from ice domes covering these areas, Scandinavia and the Barents Sea. Both reconstructions also show a southward-moving ice shelf over Baffin Bay, David Strait and the Labrador Sea, fed from the Laurentide, Innuitian and Greenland ice domes and nearly grounded in Davis Strait. The North Atlantic margin of the combined ice shelf extended in an arc from the Grand Banks off Newfoundland to the continental shelf off Scotland, coinciding approximately with the August 0 °C sea-level isotherm 18,000 yr ago[1]. This margin ablated largely by iceberg calving.

The importance of ice shelves to late-Würm glaciation is the recognition that a huge unbroken ice cover existed over polar seas and adjacent portions of North America, Greenland and Eurasia; that this ice cover constituted a unified Arctic Ice Sheet with grounded terrestrial and marine portions and floating ice shelves; and that all components were interconnected dynamically, each depending for its existence on the others. Late-Würm glacial history can be understood by viewing the Arctic Ice Sheet as one dynamic system, stressing the predominantly marine character of this system and the dynamics of marine ice domes in relation to ice streams, ice shelves, and calving bays. The huge interior floating ice shelves were key factors in stabilising the Arctic Ice Sheet, for they buttressed marine portions which in turn abutted terrestrial portions.

Ice domes

Our proposed Arctic Ice Sheet consisted of marine domes over Hudson Bay, the Gulf of Bothnia, the Barents Sea, the Kara Sea, the Queen Elizabeth Islands and possibly the East Siberian Sea; and terrestrial domes over the Canadian Cordillera, Newfoundland, Greenland, Iceland, the British Isles and the Putorana Plateau.

Seaward spreading from major domes of the Arctic Ice Sheet differed fundamentally from landward spreading: (1) It occurred primarily in marine portions over a bed that had been mostly below sea level, and which thus remained mostly thawed, whereas landward spreading occurred primarily in terrestrial portions over a bed that had been mostly above sea level and which had been and partly remained frozen. (2) Seaward spreading was primarily inward convergent flow from ice divides joining the domes, and thus accelerated more rapidly than landward spreading, which was primarily outward divergent flow from the ice divides. Because basal frictional heat increases as ice accelerates, more meltwater was formed at the bed for seaward than for landward spreading. (3) Seaward spreading ended as rapid stream flow because ice moved between coastal mountains and islands; landward spreading ended as slow sheet flow because ice moved over broad interior plains. Ice-sheet energy was thus focused on narrow zones along the seaward margin, but was dispersed across broad fronts along the landward margin. These features combined to increase ice-bed uncoupling, the primary cause of glacial surges, for seaward spreading compared with landward spreading. Surge conditions would thus develop first in ice streams and outlet glaciers draining marine portions of the Arctic Ice Sheet.

Ice streams

Mountain ranges along continental margins follow the landward side of the continental shelf and commonly project through an ice-dome margin. Hence, ice flowing seaward from the terrestrial portions of an ice dome commonly funnels into outlet glaciers that erode fjords through coastal mountains, and focus ice drainage in ice streams that erode troughs on the continental shelf. These ice streams are also supplied by the grounded marine portions of the ice dome. Ice-stream troughs are fore-deepened, as are fjords, so that a low submarine sill usually exists at the outer margin of the continental shelf where ice streams become afloat and merge with ice shelves. The sills and ice shelves combined to stabilise ice-stream grounding lines at many continental-shelf margins during the late-Würm. When ice-stream grounding lines retreated across these sills, they swept over the entire continental shelf and migrated into outlet-glacier fjords. We conclude that the ice streams draining the Arctic Ice Sheet were in stable equilibrium where they began as outlet glaciers, unstable equilibrium where they extended across continental shelves, and metastable equilibrium where they merged with floating ice shelves (Fig. 3). Constraints by thick ice shelves caused compressive flow, ice thickening and divergence where ice streams became afloat. This stabilised ice shelf–ice stream grounding lines at sills and bottlenecks associated with submarine banks along the outer continental-shelf margin. As soon as ice streams were able to punch through retreating ice shelves, however, the extending flow, ice thinning and convergence at the heads of ice streams became unchecked and caused grounding-line recession. The inherently unstable marine portions of the ice dome were then transformed into ice shelves and carved up by calving bays (Figs 3 and 4).

Mountains and islands along ice dome–ice shelf junctions in Antarctica are commonly unglaciated because outlet glaciers and ice streams tend to channel flow around elevated coastal topography. In our reconstruction, this implies that unglaciated portions[18,19] of Banks Island and Baffin Island, for example, are not indicative of thin inland ice, but result from channelling of sheet flow into fjords and straits, which allowed unglaciated highlands and headlands to exist along the ice dome–ice shelf junction. Ice streams similarly drained ice around Devon Island and Newfoundland, where local ice domes may have existed.

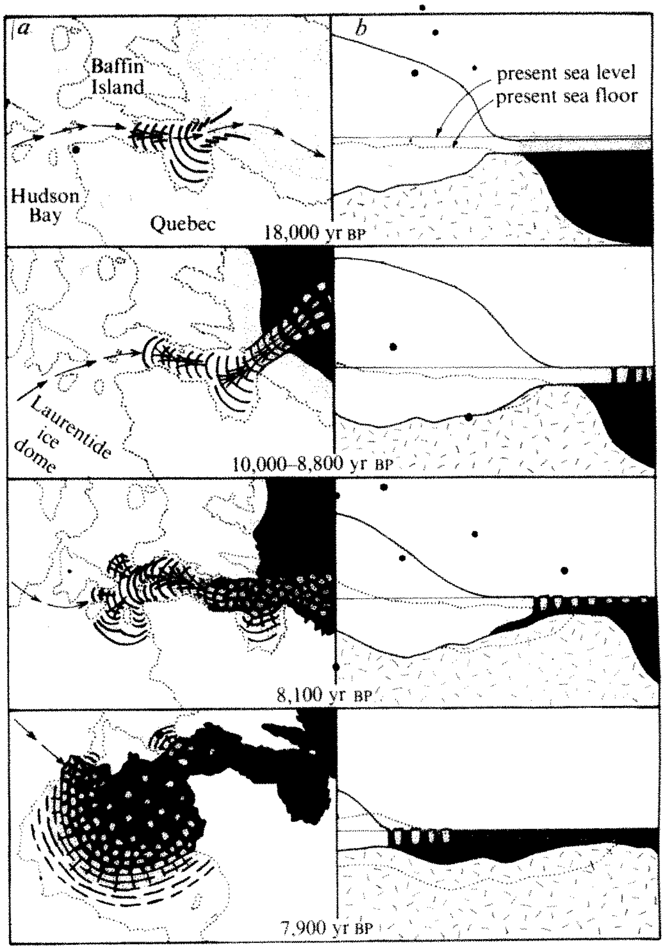


Fig. 4 Migration of a calving bay up the Hudson Strait ice stream and collapse of the Laurentide ice dome over Hudson Bay. Successive stages are shown in the plan (*a*) and profile (*b*) pairs, where the profile is taken along the ice flowline (→) shown in plan. White areas denote the grounded ice sheet, dotted areas denote the floating ice shelf and tabular icebergs, black areas denote seawater, and checked areas denote bedrock. In *a*: ...., present shorelines; ⌒ schematic crevasse patterns based on Fig. 3. The Hudson Strait ice stream was stabilised by an ice shelf in the Labrador Sea 18,000 yr ago. By about 8800–10,000 yr ago this ice shelf had retreated and thinned sufficiently for the ice stream to punch through it between the longitudinal-shear crevasses along its floating length so that a calving bay followed the ice stream grounding line up Hudson Strait. The Laurentide ice dome collapsed in a few centuries after the grounding line and calving margin of the surging ice stream crossed the island pinning points at the entrance to Hudson Bay.

Ice shelves

What maintained the stability of the large floating ice shelves that may have been necessary to prevent ice streams from drawing down marine portions of the Arctic Ice Sheet? One requirement is that a positive mass balance existed over all but calving and melting margins of the ice shelves. In the Arctic Ocean a positive annual mass balance would have been expected once northward heat transport from the North Atlantic Current essentially ceased[20] during the late-Würm maximum, when the North Atlantic Current flowed eastward towards Africa rather than north-eastward into the Norwegian Sea[21]. This change in current direction caused lower North Atlantic sea-surface temperatures so that they were compatible with an ice shelf that had a snowline close to sea level along its North Atlantic calving margin.

A second requirement is that snow accumulation over the Arctic Ice Sheet was sufficient to maintain the iceberg calving rate, which can be estimated from present Antarctic ice shelves, along the North Atlantic ice-shelf margin. The nearly constant forward velocity about 100 km behind the calving margin of the Ross Ice Shelf is about 0.9 km yr^{-1} (ref. 22), and seems to

Table 1 Ice-accumulation rates on the Arctic Ice Sheet necessary to maintain a 900 m yr^{-1} iceberg calving rate in the North Atlantic

	Minimum ice sheet (Fig. 1)	Maximum ice sheet (Fig. 2)
Hudson Strait		
Mean depth below 100 m (m)	243	243
Width over 100 m deep (km)	70	70
Area below 100 m deep (km^2)	17	17
Mean annual ice discharge (km^3 yr^{-1})	15	15
Area of accumulation basin (km^2)	0.55×10^6	0.55×10^6
Mean accumulation in basin (m yr^{-1})	0.027	0.027
Davis Strait		
Mean depth below 100 m (m)	300	300
Width over 100 m deep (km)	300	300
Area below 100 m deep (km^2)	90	90
Mean annual ice discharge (km^3 yr^{-1})	81	81
Area of accumulation basin (km^2)	2.36×10^6	2.36×10^6
Mean accumulation in basin (m yr^{-1})	0.034	0.034
Denmark Strait		
Mean depth below 100 m (m)	171	171
Width over 100 m deep (km)	350	350
Area below 100 m deep (km^2)	60	60
Mean annual ice discharge (km^3 yr^{-1})	54	54
Area of accumulation basin (km^2)	0.34×10^6	0.84×10^6
Mean accumulation in basin (m yr^{-1})	0.159	0.064
Iceland-Faeroe Ridge		
Mean depth below 100 m (m)	316	316
Width over 100 m deep (km)	380	380
Area below 100 m deep (km^2)	120	120
Mean annual ice discharge (km^3 yr^{-1})	108	108
Area of accumulation basin (km^2)	3.76×10^6	10.84×10^6
Mean accumulation in basin (m yr^{-1})	0.028	0.010
Faeroe-Shetland Trench		
Mean depth below 100 m (m)	333	333
Width over 100 m deep (km)	210	210
Area below 100 m deep (km^2)	70	70
Mean annual ice discharge (km^3 yr^{-1})	63	63
Area of accumulation basin (km^2)	0.72×10^6	0.72×10^6
Mean accumulation in basin (m yr^{-1})	0.088	0.088
North Atlantic Calving Margin		
Mean thickness 100 km from margin (m)	350	350
Width 100 km from margin (km)	1310	3800
Area 100 km from margin (km^2)	460	1330
Mean annual ice discharge (km^3 yr^{-1})	414	1200
Area of accumulation basin (km^2)	7.73×10^6	15.87×10^6
Mean accumulation in basin (m yr^{-1})	0.053	0.076

equal the iceberg calving rate because the calving margin has not undergone substantial change in this century. The nearly constant forward velocity of the Ross Ice Shelf was measured for ice averaging 350 m thick across an unsupported front 600 km wide between Roosevelt Island and Ross Island. Presumably, a forward velocity of about 0.9 km yr^{-1} would also be required 100 km behind the calving margin of the late-Würm North Atlantic ice shelf, if its forward velocity matched the estimated present Ross Ice Shelf iceberg calving rate. Sectors between pinning points along the Labrador–Baffin–Greenland–Iceland–Faeroe–Scotland line varied from 70 to 350 km wide. Ice > 350 m thick in any sector along this line would have been grounded (Table 1). Annual precipitation rates needed to maintain a 0.9-km yr^{-1} ice-shelf velocity across each sector are given in Table 1 (where the flowlines in Figs 1 and 2 are used to identify the snow accumulation basins for each sector) and are well below present rates in high polar regions, suggesting that the postulated calving rates could have been maintained easily. Hence, we think that late-Würm ice shelves existed over polar seas and stabilised the Arctic Ice Sheet.

Although the probable need to stabilise large ice streams that drained marine portions of the Arctic Ice Sheet is our primary reason for reconstructing ice shelves, other evidence is compatible with this postulate. Like present Antarctic ice shelves, our proposed Arctic ice shelves all existed in embayments nearly surrounded by large ice domes and had margins that were pinned by islands or banks. Glacial-marine sediments with very low abundance of left-coiling *Globigerina pachyderma* deposited in the Norwegian and Greenland seas during the late-Würm maximum indicate absence of the North Atlantic Current and presence of a complete ice cover[23]. Late-Würm sea-surface temperatures in the North Atlantic were compatible with an ice-shelf calving margin between Newfoundland and Scotland[1]. Nothing yet reported from the Arctic Ocean floor precludes existence of a late-Würm ice shelf; whereas slow sedimentation rates, striated clasts and truncated rises on this ocean floor are compatible with a debris-carrying ice shelf slowly ablating on its underside and scraping across pinning points[24–26].

Calving bays

We postulate that dynamic processes involving ice streams, ice shelves and calving bays were essential factors in disintegration of marine portions of the Arctic Ice Sheet. Calving bays have an important role in removing ice rapidly, and exist in metastable equilibrium. That is, they are in stable steady state for small perturbations that do not permit ice-shelf grounding lines to migrate over bedrock sills or ice-shelf calving margins to retreat from pinning points, but are in unstable catastrophic retreat for large perturbations that allow either of these events to occur. Hence, calving bays can remove floating ice shelves, or can disintegrate grounded marine ice by propagating up surging ice streams that have punched through buttressing ice shelves (Figs 3 and 4).

A view of Northern Hemisphere deglaciation that involves these dynamics is now possible. The Arctic Ice Sheet underwent peripheral fluctuations between about 22,000 and 14,000 yr ago. It then suffered step-by-step collapse of its marine portions concurrently with slow ablation of its terrestrial fringes. First, rising air temperatures and northward migration of the North Atlantic Current ablated the North Atlantic ice-shelf calving margin from pinning points between Iceland and Scotland shortly before 14,000 yr ago[21], causing ice-shelf recession into the Norwegian Sea. Late-Würm Scandinavian ice had extended to the edge of the Norwegian continental shelf, where it terminated in water that was commonly up to 450 m deep[27,28]. The topography of the Norwegian continental shelf[27,28] indicates to us that outlet glaciers from the Scandinavian dome continued across the continental shelf as ice streams up to 250 km long. If so, we would argue from the viewpoint of ice stream–ice shelf dynamics that the Norwegian Sea ice shelf buttressed these ice streams, and ice streams from the Norwegian Trench, the Barents Sea dome, and the Icelandic dome. In this case, the broad Egga moraines would have been deposited at the ice dome–ice shelf junction. Ice-shelf recession into the Norwegian Sea about 14,000 yr ago would have unbuttressed the ice streams, causing surges and resulting in rapid removal of marine ice from adjacent continental shelves by calving bays. This is compatible with the distribution and age of the oldest late-Würm moraines on the outer Norwegian and Icelandic coasts[29–32]. Whether the Barents Sea dome receded at this time is unclear. Shell ^{14}C dates from emerged marine deposits imply that major recession of Barents Sea ice occurred relatively late[33], which is compatible with ^{14}C dates of the Marchida moraines in north-western USSR that place final disintegration of eastern Barents Sea ice in Pre-Boreal time[34]. New dates on shells from the westernmost coast of west Spitsbergen, however, if they are reliable, suggest that initial deglaciation of western Barents Sea ice may have been earlier than previously expected (G. Boulton, personal communication, 1975). In any case, we postulate that subsequent north-westward recession of the Norwegian Sea calving bay into the Greenland Sea would have removed grounded ice from the East Greenland continental shelf. Finally, the heart of the Scandinavian ice sheet was removed by a calving bay that migrated up the region now covered by the Baltic Sea and the Gulf of Bothnia. The Gulf of Bothnia was deglaciated by 9300–8900 yr ago[35,36].

Late-Würm climatic amelioration also caused the ice shelf over the Arctic Ocean to unground north of Alaska and to retreat toward Siberia and Greenland, resulting in step-by-step surges of northern Laurentide, Siberian and Innuitian ice streams. Deglaciation of northern Laurentide ice probably occurred largely by calving bay propagation up these surging ice streams. It had begun by 12,400 yr ago on north-western Victoria Island[37]; by 10,500 yr ago in Dolphin and Union Straits, and by 10,200 yr ago in Coronation Gulf. As late as 10,000 yr ago, however, a Laurentide–Innuitian ice lobe still occupied Viscount Melville Sound. Ice recession occurred in Parry Channel between the Laurentide and Innuitian domes by 9500–9000 yr ago, allowing deglaciation of McClintock Channel and Peel Sound by 9200 yr ago[38–40].

Recession of westernmost Innuitian ice from Prince Patrick Island had begun by 11,700 yr ago and from Melville Island by 11,300 yr ago. Deglaciation of the inner channels under the Innuitian dome occurred 10,000–9000 yr ago[40]. Meanwhile, the Labrador Sea calving bay had migrated past pinning points in Davis Strait and retreated into Baffin Bay by 10,000–9000 yr ago[41–45], then up Hudson Strait, past the island pinning points at the north end of Hudson Bay, and gutted out the remainder of the Laurentide ice dome about 8100–7900 yr ago (Fig. 4). Finally, a calving bay migrated into Foxe Basin shortly after 7000 yr ago[46]. The hearts of most major domes of the Arctic Ice Sheet were thus removed, in some cases leaving terrestrial fringes to ablate by melting. The Greenland dome still remains in existence, because it alone among the major domes was largely terrestrial and therefore immune to irreversible disintegration mechanisms affecting marine ice domes.

Conclusions

We believe that the concept of an Arctic Ice Sheet harmonises geological and glaciological problems associated with standard reconstructions. It fits the pattern of deglaciation and reconciles the problem of initial major recession of peripheral terrestrial portions as opposed to later major retreat of interior marine portions. It conforms with the dynamic relationship of grounded marine ice to ice streams and ice shelves that we postulate for the present marine West Antarctic ice dome[7–9]. Moreover, it emphasises that the Arctic Ice Sheet consisted of marine portions in unstable equilibrium confined between land portions in stable equilibrium and ice shelves in metastable equilibrium. Perturbations of stable sectors were reversible, perturbations of unstable sectors were irreversible, and perturbations of metastable sectors were reversible if small, but irreversible if large. The critical perturbations during the last 14,000 yr were intervals of climatic warming that affected the margins of the Arctic Ice Sheet. These perturbations were reversible along terrestrial margins in stable equilibrium, but were large enough to be irreversible along ice-shelf margins in metastable equilibrium. Terrestrial portions retreated slowly across wide fronts by melting. Marine portions that had been in unstable equilibrium collapsed by rapid inroads of calving bays which migrated up surging ice streams after climatic warming removed the confined and pinned ice shelves which had prevented ice-stream surges. Cryosphere–hydrosphere interactions thus destroyed the marine heart of the Arctic Ice Sheet, whereas cryosphere–atmosphere interactions eliminated terrestrial fringes.

Implicit in our Arctic Ice Sheet hypothesis is the suggestion that ice shelves formed before marine portions of the ice sheet. Otherwise, these marine portions, being inherently unstable, might never have existed. The implications of this suggestion will be presented in a later publication.

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DNA sequences coding for the H2B histone of *Psammechinus miliaris*

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Starting from restriction enzyme cleavage sites of known topologies the DNA sequences coding for two thirds of the H2B histone protein, together with some 3′ extracistronic sequences, have been determined for the sea urchin Psammechinus miliaris. *This unambiguously identifies this gene and further reveals its 5′–3′ polarity and accurate map position in a cloned histone DNA repeat unit.*

THE coding sequences of the five histone proteins are arranged[1] in the order H4, H2B, H3, H2A and H1 in a 5.6–6 kb DNA repeat unit[1,2] which is many hundred times repeated on the chromosomal DNA of *Psammechinus miliaris*[3], the first species for which the exact gene order[1] and the direction of transcription[4] unravelled. It now seems that an identical gene arrangement is found for *Strongylocentrotus* histone DNA[5,6], while unpublished work from Hogness' laboratory and our own (Jean Burckhardt) suggests that the histone genes in *Drosophila* are organised differently. Here we report the base sequence arrangement of two sections of the H2B coding sequence of the histone DNA isolated[7] from the λ-histone phage λh22 with essentially three aims in mind.

(1) To test the identification and map position of the H2B coding sequence, formerly obtained by hybridisation of the putative H2B mRNA to histone DNA[8].
(2) To verify the polarity of the H2B gene, and hence of the gene cluster, as previously determined from a combination of resection with 5′ exonuclease and hybridisation with individual mRNAs to histone DNA[4].
(3) To confirm the accuracy of partial denaturation mapping of genes and spacers in the electron microscope[2].

These studies will also enable us to gain new insights into the evolution of the H2B proteins and to assess the fidelity of sequence conservation during maintenance and amplification of histone DNA cloned in a lambda vector.

Sequencing procedures

Both the sequencing techniques[9,10] used for these experiments depend on the availability of restriction cleavage sites as unique starting points for the actual sequence analysis.

Figure 1*a* depicts the structural map of the *Psammechinus* histone gene cluster as it was compiled from biochemical[11] and electron microscopic[2] analyses. The histone DNA, reclaimed from λh22 accommodates five (possibly six; see below) cleavage sites for the restriction enzyme *Hin*dII, as indicated on the map. The fragments A, C and F produced by this treatment can be further cleaved asymmetrically by digestion of the DNA with restriction enzymes *Hae* II and *Bgl* II (see Fig. 2). Two of these restriction fragments (fragments 2 and 4) terminate in close proximity to one another in the DNA segment predicted to contain the H2B sequences. The termini of these DNA fragments, with their well defined locations within the histone DNA provide suitable starting points for the sequencing of the H2B gene. Moreover, fragment B can be strand separated by gel electrophoresis[9] and subjected to sequence analysis.

Histone DNA (25 μg), prepared from λh22 recombinant DNA by cleavage with *Hin*dIII followed by purification from λDHA by two cycles of actinomycin CsCl density gradient centrifugation[12] was digested with 50 units *Hin*dII enzyme for 3 h in 50 μl buffer containing 20 mM Tris-HCl, *p*H 7.8; 7 mM $MgCl_2$, 7 mM mercaptoethanol. At the end of the incubation 1 μl BAP (0.06 U) was added. Incubation was continued for 30 min at

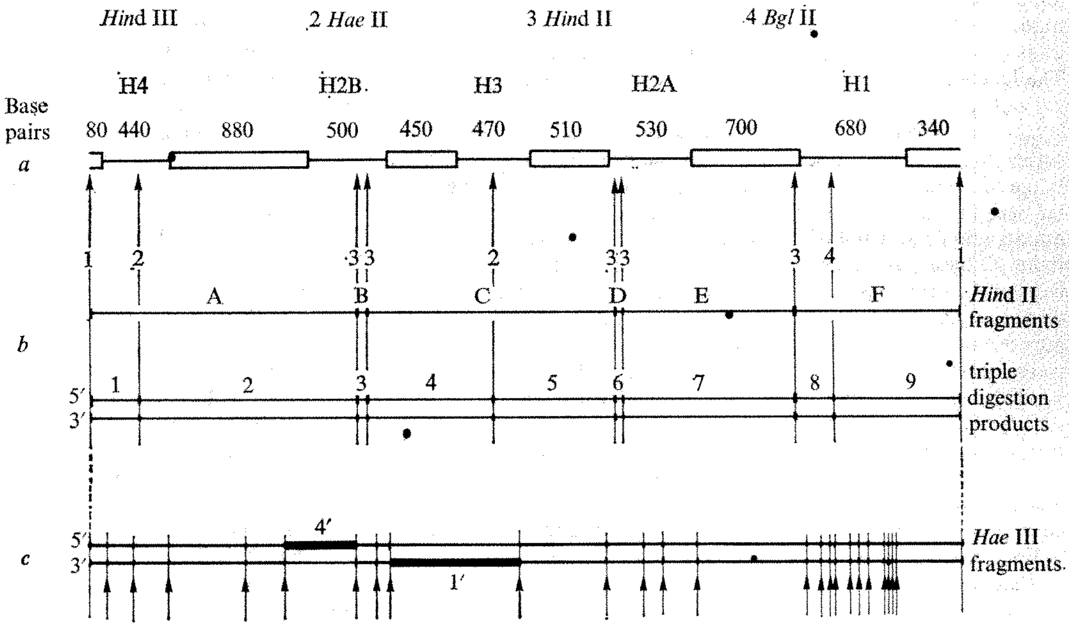

Fig. 1 Partial denaturation and restriction maps of histone DNA reclaimed from λ h22 recombinants. *a*, Distribution of spacers (double line) and histone mRNA coding sequences as determined in the electron microscope[2]. *b*, Cleavage sites for restriction enzymes *Hind* III, *Hae* II, *Hind* II and *Bgl* II (see text). *c*, Cleavage sites for *Hae* III. Fragments 2 and 4 and 1′ and 4′ were used in the sequencing experiments.

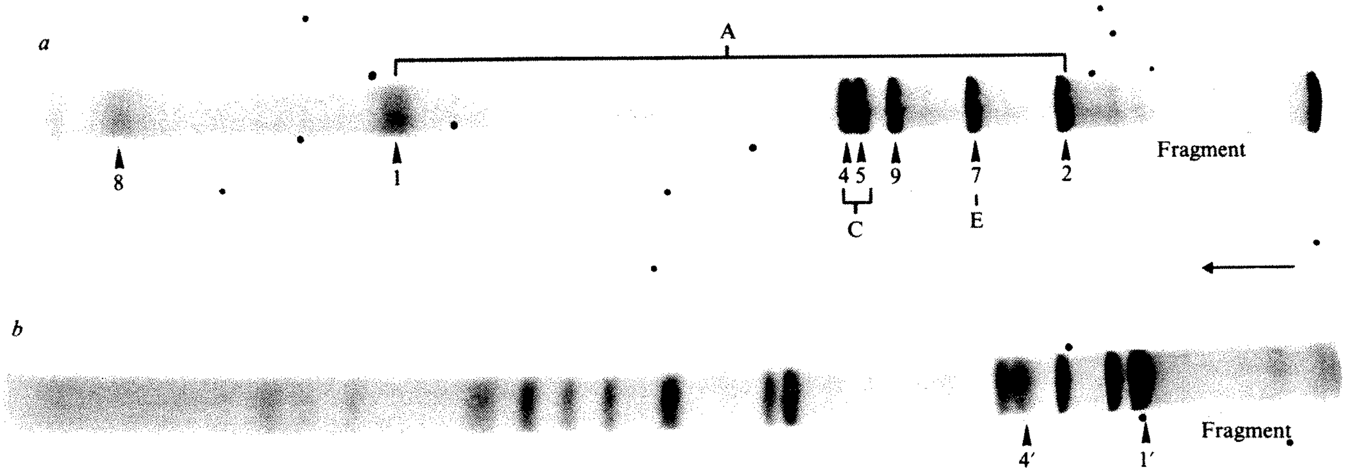

Fig. 2 Restriction fragments of cloned histone DNA h22. *a*, Electropherogramme of fragments produced after digestion of cloned histone DNA with *Hae* II, *Hin*d II and *Bgl* II. Electrophoresis was through a 4% polyacrylamide gel in 90 mM Tris; 90 mM Borate, *p*H 8.3; 2.5 mM EDTA at 4 V cm^{-1}. The fragments refer to the DNA sections shown in Fig. 1*b*. Fragments 2 and 4, labelled at only one of their termini with (γ-^{32}P)-ATP were subjected to the sequencing technique of Gilbert and Maxam[9]. *b*, *Hae* III restriction fragments of cloned histone DNA, Electrophoresis was as described above. Fragments 1′ and 4′ were recovered and annealed to strand separated γh22 DNA and served as primer for the 'plus and minus' technique[10]. For strand separation of histone recombinant phage DNA the procedure of Szybalski[14] as modified by Lozeron (W. Barnes, Ph.D. thesis) was used. One mg purified λ Sam-7 h22 (ref. 7) dissolved in 3 ml of 1 mM KH_2PO_4; 1 mM EDTA; *p*H 8.5; 0.1% sodium sarkosyl was mixed with 0.66 mg of poly (U, G) (Miles) and was denatured for 3 min at 100 °C. After quick chilling, 60 µl of 50 mM Tris-HCl, *p*H 7.6 was added and the solution made up with CsCl and water to a final vol of 16.8 ml and a density of 1.750 g ml^{-1}. The solution was transferred to a polyallomer centrifuge tube which had been previously boiled in 0.1 M EDTA, *p*H 10 and was centrifuged at 32,000 r.p.m. for 60 h at 20 °C in a Ti60 rotor. 40 fractions were collected, and the distribution of the separate DNA strands monitored either by absorbance measurements or by analytical agarose gel electrophoresis. Fractions corresponding to the heavy and the light strand were individually pooled and dialysed against 5 mM Tris-HCl, *p*H 8. Poly (U, G) was removed by incubating the samples in 0.5N NaOH at room temperature for 1 h. Tris-HCl and HCl were added to an end concentration of 0.3 M NaCl; 0.3 M Tris-HCl, *p*H 7.8 and the strands were self-annealed at 65 °C for 1 h. The DNA was then precipitated by addition of ethanol and redissolved in 300 µl 10 mM Tris-HCl, *p*H 7.5; 0.5 mM EDTA and the solution at about 0.8 mg ml^{-1} kept frozen. For one 'plus and minus' sequencing reaction, 10 µg of restriction fragment (about 1 pmol) was denatured in a boiling waterbath for 3 min, chilled in ice, mixed with about 8 µg of single stranded lambda DNA prepared as described above (about 0.5 pmol) and allowed to anneal at 67 °C for 1 h in 0.1 M NaCl; 20 mM Tris-HCl, *p*H 7.4; 10 mM $MgCl_2$, 1 mM mercaptoethanol. This material could then be used directly for elongation by DNA polymerase using the Sanger and Coulson technique[10].

37 °C. The DNA was deproteinised by shaking with phenol and was recovered by precipitation with ethanol. After washing with ethanol, the DNA was redissolved in 100 µl kinase buffer (50 mM Tris-HCl, *p*H 9.5; 0.01 M $MgCl_2$; 0.005 M DTT; 5% glycerol) to which 10 µl of (α-P^{32}-)ATP (10 µM; 1,000 Ci mmol^{-1}) made up according to the protocol of Gilbert and Maxam[9], and 1 µl T_4 kinase (0.5 U) were added. After 15 min 2 volumes of 2 M ammonium acetate were added and the DNA was precipitated with 21/2 volumes of ethanol. The DNA was recovered by centrifugation, dissolved in 100 µl 10 mM Tris-HCl, *p*H 7.8; 1 mM EDTA and loaded on a 0.2 × 15 × 40-cm 4% polyacrylamide gel made up in glycine buffer (30 ml NAOH; 30 g glycine made up to 2 l.). Electrophoresis was terminated when the unincorporated ^{32}P appeared in a lower buffer compartment. The labelled DNA bands were located by autoradiography and cut out from the gel.

The polyacrylamide gel revealed seven kinds of molecules rather than the five anticipated from the previous work[11]. The two additional cleavage products were found in the low molecular weight range. One of these, B, bridges the gap between amino acid 85 and 104 of the H2B sequence and thus is made up from 62 base pairs (see below). The nature of the other small fragment, which was only poorly labelled, has not been determined as yet. The two slowest moving components of the polyacrylamide gels corresponding to fragments A and C of Fig. 1*a* were recovered, pooled and digested with *Hae* II. This treatment yielded four DNA molecules each labelled with ^{32}P at one of their ends. Fragments 2 and 4 (see Fig. 1*a* and 2) containing the presumptive H2B DNA sequences were then subjected to the sequencing procedure of Gilbert and Maxam[9]. In addition, fragment B was recovered, denatured and strand separated by electrophoresis and the faster moving single strand sequenced[9].

For other sections of the histone DNA, the plus and minus method of Sanger and Coulson[10] was used. This procedure is based on elongation by DNA polymerase of unique primer DNA fragments hybridised to a single stranded template DNA. The availability of histone DNA cloned both in lambda[7] and in pCRI plasmid[13] proved to be very advantageous for this work. Template was obtained in preparative amounts by strand separation of histone recombinant DNA with poly(U, G) in a CsCl density gradient according to the protocol of Szybalski *et al.*[14]. Primer fragments were prepared by restriction of total recombinant pCRI h7 DNA and fractionation of the products by preparative polyacrylamide gel electrophoresis. This combination of template and primer DNA each obtained using different vectors eliminated any possible vector DNA background during sequencing.

For the preparation of primer DNA, 600 µg of the recombinant plasmid pCRI (Pm histone) 7 DNA[13] were cleaved with *Hae*III restriction endonuclease (a gift of Dr Nigel Brown) and the resulting fragments were fractionated on a 0.3 × 20 × 45 cm, 5% polyacrylamide slab gel[9]. Then 2 µg of reclaimed 6kb *Psammechinus* histone repeat, digested with *Hae* III, were run in parallel slots in order to identify the histone-specific DNA fragments. The gel was immersed for 1 h in electrophoresis buffer containing ethidium bromide 0.5 µg ml^{-1}. Histone DNA fragments were excised under ultraviolet light. The DNA was recovered by electrophoresis into a dialysis bag, taken up in 0.3 M sodium acetate, *p*H 7, mixed with 21/2 volumes of ethanol, kept at −20 °C overnight and spun down in a SW50 rotor at 45000 r.p.m. for 1 h at 4 °C. The DNA fragments were redissolved in 200 µl 10 mM Tris-HCl, *p*H 7.5; 0.5 mM EDTA and kept frozen until use.

The *Hae* III fragments have been mapped (see ref. 11, and Figs 1*c* and 2). The largest of these (1′) includes the spacer between the H2B and the H3 gene and part of the latter. This DNA section and 9 other *Hae* III DNA fragments were isolated (see above) and on denaturation were individually annealed to either the light or the heavy strand of λh22 DNA known to contain the coding and the anti-coding sequence of the histone

	60					65					70				
	Tyr	Lys	Val	Leu	Lys	GlN	Val	His	Pro	Asp	Thr	Gly	Ile	Ser	Ser
5'	TAC	AAA	GTT	CTC	AAG	CAG	GTT	CAC	CCT	GAC	ACT	GGT	GTC	TCC	AGC
3'	ATG	TTT	CAA	GAG	TTC	GTC	CAA	GTG	GGA	CTG	TGA	CCA	CAG	AGG	TCG
	Tyr	Lys	Val	Leu	Lys	Gln	Val	His	Pro	Asp	Thr	Gly	Val*	Ser	Ser
	Tyr	Lys	Val	Leu	Lys	Gln	Val	His	Pro	Asp	Thr	Gly	Ile	Ser	Ser
				45					50					55	

	Hae III site									HindII site				
75					80					85				89
Arg	Ala	Met	Ser	Val	Met	AsN	Ser	Phe	Val	AsN	Asp	Val	Phe	Glu
													(T)	
CGG	GCC	ATG	ACA	ATC	ATG	AAC	AGC	TTT	GTC	AAC	GAT	ATC	ATC	GAG
GCC	CGG	TAC	TGT	TAG	TAC	TTG	TCG	AAA	CAG	TTG	CTA	TAG	TAG	CTC
													(A)	
Arg*	Ala	Met	Thr*	Ile*	Met	AsN	Ser	Phe	Val	AsN	Asp	Ile*	(Ile*)	Glu
Lys	Ala	Met	Gly	Ile	Met	AsN	Ser	Phe	Val	AsN	Asp	Ileu	Phe	Glu

90					95					100				
Arg	Ile	Ala	Gly	Glu	Ala	Ser	Arg	Leu	Thr	Ser	Ala	Asn	Arg	Arg
AGC	ATC	GCC	GGC	GAA	GCT	TCC	CGT	CTC	ACC	CAG	TAC	AAC	AAG	A(A)
TCG	TAG	CGG	CCG	CTT	CGA	AGG	GCA	GAG	TGG	GTC	ATG	TTG	TTC	T(T)
Arg	Ile	Ala	Gly	Glu	Ala	Ser	Arg	Leu	Thr*	Gln*	Tyr	Asn	Lys	(Lys)
Arg	Ile	Ala	Gly	Glu	Ala	Ser	Arg	Leu	Ala	His	Tyr	Asn	Lys	Arg
			75					80					85	

	HindII site														
	105					110					115				
	Ser	Thr	Val	Ser	Ser	Arg	Glu	Ile	GlN	Thr	Ala	Val	Arg	Leu	Leu
	TC														
G	TCA	ACX	ATC	AGT	AGC	CGG	GAG	ATT	CAG	ACC	GCC	GTG	CGC	CTT	CTT
C	AGT	TAY	TAG	TCA	TCG	GCC	CTC	TAA	GTC	TGG	CGG	CAC	GCG	GAA	GAA
	AC														
	(Ser)	(Thr)	Ile*	Ser	Ser	Arg	Glu	Ile	GlN	Thr	Ala	Val	Arg	Leu	Leu
	Ser	Thr	Ile	Thr	Ser	Arg	Glu	Ile	GlN	Thr	Ala	Val	Arg	Leu	Leu
				90					95					100	

120					125					130				
Leu	Pro	Gly	Glu	Leu	Ala	Lys	His	Ala	Val	Ser	Glu	Gly	Thr	Lys
CTC	CCA	GGA	GAG	TTG	GCC	AAG	CAC	GCC	GTG	AGT	GAG	GGG	ACC	AAG
GAG	GGT	CCT	CTC	AAC	CGG	TTC	GTG	CGG	CAC	TCA	CTC	CCC	TGG	TTC
Leu	Pro	Gly	Glu	Leu	Ala	Lys	His	Ala	Val	Ser	Glu	Gly	Thr	Lys
Leu	Pro	Gly	Glu	Leu	Ala	Lys	His	Ala	Val	Ser	Glu	Gly	Thr	Lys
			105					110					115	

135					140						
Ala	Val	Thr	Lys	Tyr	Thr	Thr	Ser	Arg	c-term		
										(C)	
GCA	GTG	ACC	AAG	TAC	ACC	ACC	GTC	AAG	TAA	ACGTTACAC(T)ACT	3'
CGT	CAC	TGG	TTC	ATG	TGG	TGG	CAG	TTC	ATT	TGCAATGTG(A)TGA	5'
										(G)	
Ala	Val	Thr	Lys	Tyr	Thr	Thr	Val*	Lys*	ochre	untranslated region	
Ala	Val	Thr	Lys	Tyr	Thr	Ser	Ser	Lys	c-term		
			120					125			

Fig. 3 DNA sequences of H2B histone protein. Protein sequence of H2B of *Parechinus* (top line, ref. 15) of calf thymus (bottom line, ref. 16); middle line—DNA and protein sequences of the H2B of *Psammechinus*. Amino acids in *Psammechinus* which differ from either of the other two species are marked with asterisks. Ambiguities in either DNA or protein sequences are denoted by brackets. The restriction cleavage sites relevant to the present work are boxed.

mRNAs, respectively (see Fig. 2 legend). After base sequence analysis, the DNA sequences were converted into amino acid sequences using all three possible reading frames and compared with the known amino acid sequences of the histone proteins by computer. Sequences pertaining to the H2B were identified by comparison with the known H2B protein sequence of the sea urchin *Parechinus*, determined by Strickland and von Holt[15].

Partial sequence of the H2B gene of *Psammechinus*

The H2B protein from the sperm of the sea urchin *Parechinus* contains 143 amino acids[15]. Comparison with the calf thymus protein revealed[16] that the *Parechinus* sperm protein is larger by 17 amino acids, with the additional peptide sequences appearing, in the main, at the N-terminal end of the molecule. The DNA sequences obtained from the base sequence analysis of the *Psammechinus* histone DNA were converted into amino acid sequences and recorded in Fig. 3 so as to yield maximal sequence homology with the H2B protein of *Parechinus* (top line) and calf thymus (bottom line), respectively. The numbering of the amino acids of the *Psammechinus* protein was made to conform with that of *Parechinus*. Starting at the 5′ terminus of fragment 2 (Fig. 1) the Gilbert and Maxam procedure yielded about 75 bases of the coding sequence, from approximately amino acid 84 to amino acid 60. Starting from the labelled *Hin*dII site of fragment 4 a sequence of 80 nucleotides was obtained, representing the anti-coding sequence ranging from amino acid 107 up to 131 of the H2B protein. In this experiment, the first two or three bases were lost from the analysis so that the known sequence started at Val (position 107). The position of the *Hin*dII cleavage site may be predicted from the amino acid sequence of the protein, as indicated in Fig. 3. The fast-moving single strand of B yielded the coding sequences for codons 104 to 90, leaving a gap of a few amino acids unaccounted for.

Comparison of the H2B amino acid sequences and the DNA sequence obtained from hybridisation of one of the smaller *Hae* III fragments (indicated in Fig. 1*c* as a bold line) to the light strand of λh22 DNA and subsequent sequence analysis using the plus and minus method[10] showed that this section contained the missing DNA sequence between amino acid positions 85 and 90. The first readable codon specified Ser in position 82 and could be extended up to amino acid 91. Here too, there was a small overlap in DNA sequence in both sets of experiments. From the position of the first readable codon 82 on the electrophoretic gel relative to the dye marker it was predicted that the *Hae* III sequence which had served as a primer for strand extension, was to be found some 18 base pairs to the left of codon 82. As can be seen from the first set of data, it lies at the predicted point between Arg 75 and Ala 76.

From the mapping experiments (Fig. 1*c*) it was also predicted that the large *Hae* III fragment used in these experiments encompasses most of the spacer region between the H2B and H3 gene, with a *Hae* III site just to the right of the former. Strand extension starting from the fragment annealed to the heavy λh22 strand was expected to include the coding sequence of the C-terminal end of the H2B protein. Comparison of the DNA and protein sequences has confirmed this. The sequence contains, in addition to a short stretch of DNA, presumably coding for an untranslated region of the H2B mRNA, about 45 readable bases extending to His 127. Here again there is a small overlap of 15 bases with the previous set of data. Our data then, covering the proteins sequences from amino acid 60 to 143 provide an unequivocal identification of the H2B gene confirming our results previously obtained by the classical methods of mRNA translation *in vitro*[8] and RNA–DNA hybridisation[1].

A small number of ambiguities in the DNA sequences and hence in the amino acid assignments remain. These have been noted in Fig. 3.

Polarity and map position of the H2B coding sequence

The positions of the *Hin*dII and the *Hae* II cleavage sites have been determined independently from overlaps found in complete digests using a variety of restriction enzymes or by our partial enzyme digestion procedure (Fig. 1). In these experiments it was established that fragment A is cleaved asymmetrically by *Hae* II so that the terminus on the left of the map becomes associated with the small DNA fragment 1 and the other terminus with fragment 2. Moving from the ^{32}P-labelled 5′ end of fragment 2 towards the left (Fig. 1*a*), a partial sequence of the H2B coding sequence was obtained. In turn, the ^{32}P-labelled 5′ terminus of fragment 4 yielded the anti-coding sequence of the H2B from amino acid 107 to 131. Also, *Hae* III fragment annealed to the light and another small fragment annealed to the heavy λh22 DNA yielded upon extension of the 3′ termini with polymerase anti-coding and coding sequences of the H2B, respectively. Together, these experiments prove unequivocally that the lower strand (Fig. 1*b* and *c*) includes the coding sequence and hence these experiments confirm that transcription of H2B is from the left to right of the map—as shown for the histone DNA repeat unit by exonuclease experiments[4].

The present work can also be used to place the H2B coding sequence accurately in the partial denaturation map of cloned histone DNA. Electron microscope mapping studies revealed that the *Hin*dII enzyme cleaves the double-stranded DNA region presumed to represent the H2B gene[2] in the right half of that DNA segment. Our present work shows that there are in fact two *Hin*dII sites in close proximity to one another at positions 85 and 105, about two thirds of the way along the H2B sequence. The *Hae* III sites predicted to lie in the same area (see Fig. 1*c*) have now been mapped, by sequencing, near Ala 76 and 125, respectively. It should be mentioned, that the largest *Hae* III fragment has been shown, by sequence analysis, to extend into the H3 coding sequences to the right. Furthermore, the N-terminal end of the H2A coding sequence has been located using another *Hae* III fragment of known map position, so that this coding sequence also has been identified and mapped unequivocally within the cloned histone DNA of *Psammechinus* W. S. and M. B., unpublished). Our sequencing data therfore confirm our previous assignments of genes[1,7] as well as their positions in relation to spacer DNA[2]. Nothing can as yet be said about the extracistronic sequences since they show no homologies to known untranslated mRNA sequences.

Evolution of the H2B protein sequences

The available DNA sequences define about two thirds of the amino acid moieties of the H2B protein of *Psammechinus*. There is extensive sequence homology with both the calf thymus and the sea urchin *Paraechinus* H2B protein, especially near the C terminus. This is in keeping with the general tenet that histones are conservative in evolution. There are some disparities, however. Thus position 78 and 100 are different in all three proteins. Two substitutions Arg⇌Lys occur in positions 75 and 143. Val in position 142 apparently is different from the Ser in either of the two other proteins. There is a whole series of interchanges involving Val⇌Ile. It should be noted that, with two exceptions, all amino acid substitutions are conservative and must therefore be the result of natural selection acting at the level of protein sequence within the living animal. This suggests that the change we observe reflects the evolution of the H2B sequences within the sea urchin rather than a randomisation process which might have occurred during cloning and amplification of the histone DNA in a lambda vector, and further supports the hypothesis that the cloned histone DNA has remained essentially unaltered after passage through *E. coli*.

We have asked whether the frequent substitutions Val⇌Ile could be neutral mutations occurring by a random process. In this context it is pertinent to note that the codons in region 62, 66, 72, 79, 84, 87, 91, 107 and 112 are such that interconversion of these two amino acids could occur by substitution of a single base G⇌A. If the appearance of A or G in the first position was determined purely by chance, there would be a 1 : 8 probability of finding only Val at a given position in all three species, a 1 : 8 chance of finding only Ile and a 6 : 8 probability of finding a mixture of the two. In the nine instances cited above the frequency of occurrence of Val only, Ile only or Val and Ile mixed at a given site in the three species are 3 : 9, 2 : 9 and 4 : 9, respectively. These ratios are distinctly different from those expected from a stochastic process and the appearance, in many instances, of Val only, or Ile only, must be the result of natural selection at the protein level.

Considering only amino acids which have been unequivocally identified, the known sequence of *Psammechinus* differs from that of *Parechinus* in overall nine moieties, but only in seven from calf thymus H2B; while the work of the von Holt group[15] has shown the calf thymus and *Parechinus* sperm H2B to differ in 11 amino acids within the sequences delimited by the present work. The known *Psammechinus* H2B sequences are more like those of calf thymus H2B than those of *Parechinus* sperm. Now, the H2B histones of sea urchins are a diverse class of proteins giving rise to variants[17] which are often associated with tissue-specific gene expression. Interestingly, H2B proteins isolated from *Parechinus embryos* are more like calf thymus H2B (von Holt, personal communication) and so it is possibile that the histone DNA clone we are analysing is derived from those gene units which would normally have been active during early stages of sea urchin development.

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letters to nature

A galactic vacuum cleaner?

A REMARKABLE feature of the distributions of CO, H_2, HI, HII and dust in the Galaxy is that their most dense regions form a broad ring-like structure surrounding the galactic centre, along with a dense nuclear disk located at the centre[1–4]. Between the ring and the nuclear disk there exists a region of very low density. The radius of the nuclear disk is approximately 600 pc, and the ring extends from an inner radius of 2–4 kpc out to a radius greater than 15 kpc for HI, but to only 8–10 kpc for the interstellar components expected to be generated by spiral shocks (namely CO, H_2 and HII). We point out here that this structure is a possible consequence of a postulated stellar bar at the centre of the galaxy.

The difference between the HI ring and that of the other components can be explained in terms of the radial variation of the spiral shock strength, and this idea forms the basis of Stecker's recent proposal for modifying the galactic population classes[5]. The shock strength is an increasing function of the component of the gas velocity perpendicular to the assumed spiral stellar potential, namely $r(\Omega-\Omega_p)\sin i$, where r is the galactocentric radius, Ω and Ω_p are the angular velocities of the gas and spiral pattern respectively, and i is the spiral pitch angle. One way in which the shock strength could decrease to zero at 8–10 kpc, and hence explain the confinement of CO, H_2 and HII to within that radius, is for corotation ($\Omega = \Omega_p$) to occur at around 10 kpc. This would require $\Omega_p \approx 25$ (km s^{-1}) kpc^{-1} on the basis of the Schmidt model, and some support for such a value for Ω_p comes from the shape of the HI line profile in the direction of the anticentre[6].

Shu[7] has suggested an explanation for the absence of HII regions in the 600 pc–3 kpc region assuming that the inner Lindblad resonance occurs at about 3 kpc. Standard spiral density wave theory predicts that there should be no stellar density wave inside this radius, and therefore no shocks to produce the HII regions and so on. But although this could explain the deficiency of shock-generated species it does not explain why all the interstellar material is deficient there.

Here we propose an alternative explanation for the distribution of interstellar matter in the central region on the basis of a hypothetical stellar bar situated at the centre of the Galaxy. Sorensen, Matsuda and Fujimoto[8] have computed the flow of gas in barred spiral galaxies, assuming a specified potential due to the stellar bar plus a stellar disk, and neglecting the self gravity of the gas. The results of these calculations show that two pairs of shocks are formed. One pair are straight, almost radial shocks situated on the leading side of the bar (B in Fig. 1), and the other pair are trailing spiral shocks originating near the bar–gas corotation points (S in Fig. 1). The S shocks can be identified with the trailing spiral arms in barred galaxies, while the B shocks are sometimes observed as straight, bisymmetric dark lanes within the stellar bar[9].

The most important aspect of the B shocks as far as the present considerations are concerned is that they induce a strong inflow of gas, so that almost all the gas originally occupying the region within the corotation points is swept into a dense nucleus at the centre of the bar. The physical reason for this is that the gas plunges into the B shocks almost at normal incidence leading to a very strong shock (in contrast to the weaker shocks in ordinary spirals with oblique incidence). The gas loses both angular momentum and energy (by radiation), and consequently falls towards the centre after the shock, slowly spiralling inwards as it successively traverses the shocks. The gas flow through the S shocks is more complicated with infall produced in the inner part of the shock while outside the corotation point the gas gains angular momentum in passing through the shock density peak and spirals outwards, although much more slowly than the infall produced by the B shocks due to the higher obliquity of incidence. In this manner the stellar bar acts as a 'vacuum cleaner' sweeping gas

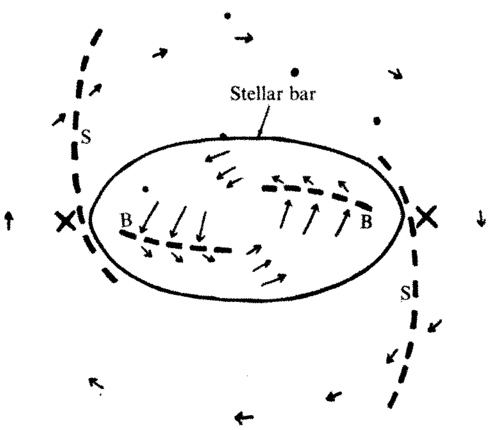

Fig. 1 Schematic diagram of the circulation of gas in a barred galaxy from the calculations of Sorensen, Matsuda and Fujimoto[8]. Arrows represent the velocity of the gas in direction and magnitude in the rest frame of the bar, the density peaks produced by the shocks are shown by the broken lines, and the crosses represent the bar–gas co-rotation points.

and dust away from the corotation region. Observations[10] of HI in barred spirals support this result in that a dense nucleus is observed at the centre of the bar along with an extended ring coincident with and extending beyond the optical spiral arms.

A small central bar at the centre of ordinarily spiral galaxies has been previously proposed in a few contexts. Mishurov and Suchkov[11] have suggested that spiral density waves may be excited by a rotating central bar, while Contopoulos[12] has shown from the calculation of stellar orbits that a gravitationally self-consistent bar can be obtained inside the inner Lindbald resonance. From the observational viewpoint attempts[13] have been made to explain the radial motions of HI at the galactic centre in terms of elliptic orbits which could possibly be induced by a bar. Observations of Cohen and Davies[14] are not inconsistent with this explanation of the radial motions, and furthermore the geometry of the HI features which they have obtained are highly suggestive of a stellar bar in that their features IIIa and XIVa would correspond to the B shocks, while features XII and I would correspond to the S shocks. If the steep drop in the interstellar densities at 2–4 kpc represents the outer extent of the in-sweeping effect of a bar then the corotation point of the gas with respect to the bar should occur at approximately this radius. This is in reasonable agreement with the extent of features IIIa and XIVa, which should lie within the corotation points, and in fact extend out to 2 kpc.

If the bar corotates with the spiral wave pattern in the outer part of the Galaxy at say 25 (km s^{-1}) kpc^{-1} then we would require a deep minimum in the rotational velocity of the gas somewhere in the region of 2 kpc from the centre in order to have a bar–gas corotation point there. This is inconsistent with the Rougoor–Oort rotation curve[15]; however the presence of strong radial motions in the central part of the Galaxy must make the definition and evaluation of the rotational velocity there uncertain, and the rotational velocities of some of the central features obtained by Cohen and Davies suggest at least the possibility of a deep minimum between 1 and 2 kpc. Such a rotational velocity minimum is observed in M31 (ref. 16), and it is noteworthy that a ring-like distribution plus a dense central nucleus for interstellar material in M31 is indicated by the combined HI and radio continuum observations.

On the other hand it is not obvious that the bar needs to co-rotate with the spiral pattern; the two may be dynamically independent with the bar rotating more rapidly than the spirals. A bar–gas co-rotation point could then be obtained at a radius of say 2 kpc even with the Rougoor–Oort rotation curve. For example, in the central part of NGC4314 tiny spirals appear which seem to be independent of the grand design[9].

In summary the presence of a small bar at the centre of our galaxy could provide a single explanation for three puzzling aspects of galactic structure, namely the strong central radial motions, HI features IIIa and XIVa, and the region between 600 pc and 2–4 kpc deficient in interstellar matter. We can estimate the mass of the bar under the assumption that it is the most massive constituent within the hypothetical bar–gas co-rotation point at approximately 2 kpc. This leads to a value less than a tenth that of the total galatic mass, which should be compared with the situation in normal barred spirals where the mass of the bar is probably a much more substantial fraction of the total mass.

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Mass distribution of massive meteoroids determined by occurrence of brilliant fireballs

DETERMINATION of the mass distribution function of bodies is of prime importance for an understanding of the evolutionary processes that determine the population of the Solar System. We report here a method for measuring mass distribution of meteoroids, by determining the occurrence frequency of very brilliant fireballs. In a certain mass regime the number distribution can be described by a power law

$$dn = (\text{const.})m^{-s}\,dm$$

where dn is the number of particles having a mass between m and $m+dm$ and s is the mass distribution index. s is a slowly varying function of mass, and values of s have been well established for meteoroid particles $10^{-12} < m \lesssim 10^{1}$ g by various techniques, and reasonable estimates are available for large bodies $m \gtrsim 10^{5}$ g (meteorites and asteroids). There is, however, a lack of information in the mass range $10^{1} \lesssim m \lesssim 10^{5}$ g corresponding[1] to meteoroids which yield meteors of visual magnitude $-2 \gtrsim M_v \gtrsim -12$ ranging from bright visual objects to very brilliant fireballs (the term fireball generally refers to any meteor brighter than Venus, $M_v \simeq -4$). The problem is one of sampling: while meteor (radar, visual, optical) counts over periods of hours at a single station are suitable for estimates of s for $m \lesssim 1$ g ($M_v \gtrsim 0$), such a procedure yields a prohibitively low count for larger objects. This difficulty would be overcome by observing over a large area of the Earth's surface for many years. We determine here the occurrence frequency of very brilliant fireballs, as a method of s measurement. A fireball of magnitude equal to that of the full Moon is an outstanding spectacle likely to be well reported in an area with a high population density. With good communications and active research groups analysing reports and recording data, a reliable estimate of the influx of such large objects could be secured. In the British Isles, extensive records of fireball events covering more than 40 yr, are available[2,3]. Most observers' estimates were based on the great illumination produced on the landscape or inside buildings, and the personnel analysing the reports were experienced workers in the field. A detailed examination of the reports reveals a consistent rate of about two lunar fireballs (apparent visual magnitude equal to or brighter than that of the full Moon) seen in the British Isles per year. Specifically, in the period 1900–36 the average occurrence frequency was 2.4 yr^{-1}. This frequency can be compared with that of visual meteors to obtain an estimate of s.

Assuming that meteor luminosity is proportional to pre-entry meteoroid mass, m, the cumulative flux N of meteors having magnitudes equal to or less than absolute magnitude, M is given by

$$\log_{10}N = (\text{const.}) - ((s-1)/2.51)M$$

For a single observer the average rate of sporadic meteors $M_v \leqslant 0$ is 0.86 h^{-1} (ref. 1), and as s is known[4] to be close to 2.35 for $+4 \leqslant M_v \leqslant -2$, the cumulative rate for $M_v \leqslant -2$ is 7.34×10^{-2} h^{-1}. Estimating the surface area viewed from the British Isles as 4×10^5 km^2, then with an area of 1.16×10^5 km^2 under surveillance by a single observer[1], the average rate for the geographical area is 2.52×10^{-1} h^{-1}.

Of the lunar fireballs during the 36-yr period, 85% were observed between 1800 and 0200 UT, with 50% occurring 2000–2300 UT (reflecting an observation bias rather than any true variation). The effective observing period is close to 8 h d^{-1}; also for this period the visual sporadic rate is close to the diurnal mean value. Although clear skies are not necessary for the detection of such brilliant objects, detection conditions depend upon the prevailing cloud type. If a cloud cover of $<7/10$ is required for detection, however, the percentage time available in the British Isles is[5] about 40%. Hence the average lunar fireball occurrence frequency becomes 2.05×10^{-3} h^{-1}. As the height of maximum luminosity is lower for brighter meteors, the apparent magnitude $M_v \simeq -12$ of a lunar fireball needs correction. From the beginning and end heights of lunar fireballs, and noting that the maximum luminosity for bright objects occurs about three-quarters of the way along the trail[7], a mean height of 60 km is found. The absolute visual magnitude (referred to a standard height of 100 km) is therefore $M_v \simeq -11$. This implies that the observed fireballs are nine stellar magnitudes brighter than meteors of $M_v = -2$.

If meteors which differ in absolute magnitude by ΔM have occurrence frequencies of N_1 and N_2, then

$$s = 1 + (2.51/\Delta M)\log_{10}(N_1/N_2)$$

leading to $s = 1.58$. The reported mean annual lunar fireball rate of 2.4 showed no systematic variation over the 36-yr period, and it seems unlikely that this value would have an uncertainty exceeding 50%. With independent sightings of a large number of fireballs, systematic magnitude errors would not be expected, and it is not unreasonable to assign an uncertainty in ΔM of ± 1 mag. The estimate of the mass distribution index, s, is thus quite good: the uncertainty in N_2 leads to an error of 0.05 in s, and the uncertainty in ΔM implies an eror of 0.06. We conclude that $s = 1.58 \pm 0.08$. The observed fireball rate is incompatible with large values of s suggested by some authors: a value of $s = 2.34$ appropriate to visual meteors implies only one lunar fireball sighting in the British Isles every 200 yr.

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Gravitational radiation from freely-falling bodies

THE problem of determining the gravitational radiation from freely-falling bodies is almost as old as general relativity itself. Investigators have deduced an energy loss, an energy gain and no radiation at all[1]. Although Eddington[2] foresaw the difficulties a half century ago, many now believe that the Einstein quadrupole formula correctly describes the rate of energy loss[3]. Cooperstock initiated a new approach to the problem by considering two bodies in an initially static configuration by virtue of an intervening strut[4]. The strut was severed and the dynamical perturbation to the gravitational field was analysed. During the stress-breaking period, the radiated energy loss agreed with the quadrupole formula. During free fall, however, the model with two point masses proved inadequate and an interior metric was required. Even then, there were indications of a breakdown of the quadrupole formula and a much larger energy flux. A model of realistic sources has now been constructed and the previous indications have been realised. The free-fall energy loss rate exceeds that which would be anticipated by the quadrupole formula by a factor of $(\alpha/\rho_0)^4$, where ρ_0 is the characteristic size of the bodies and 2α is their separation.

The model is chosen to consist of two perfect fluid spheres of radius ρ_0 with thin retaining skins. If the zero order density and pressure are

$$^{0}\varepsilon = \text{constant},\ ^{0}P = 0 \tag{1}$$

then the metric within the spheres and strut can be expressed in the form[5]

$$\mathrm{d}s^2 = \mathrm{e}^{2\nu}\mathrm{d}t^2 - \mathrm{e}^{2(\gamma-\nu)}(\mathrm{d}r^2 + \mathrm{d}z^2) - r^2\mathrm{e}^{2(\mu-\nu)}\mathrm{d}\varphi^2 \tag{2}$$

where μ is of order G^2. From the Einstein field equations, it follows that ν and γ are of the order G and G^2, respectively, in the sources, as they are in vacuum. The field equations imply that ν satisfies the Poisson equation

$$\nabla^2\nu = 4\pi G T_4^{\ 4} \tag{3}$$

From equations (1) and (3) and the conservation laws

$$\mathrm{T}^{ik};k = 0 \tag{4}$$

the first order static pressure is found.

In a short time interval, counterstresses are applied to remove the supporting strut and envelopes, and free fall begins. The metric is then described as a second and third order perturbation of the background metric (equation (2)). The second order dynamic metric is found to have the required continuity and to be singularity-free only when the complementary phenomena of stress-breaking in the strut, pressure variation in the bodies and the ensuing free-fall motion are properly synthesised. Using the field equations, it is possible to express one of the elements b' of the third order pertubation in an inhomogeneous wave equation with the first and second order fields and the dynamic energy–momentum tensor T^{ik} as sources. This lengthy equation may be expressed symbolically in the form

$$\Box\, b' = (\mathrm{T}) + (\nu)(b) \tag{5}$$

where (T) represents terms which are linear in components of the energy–momentum tensor and $(\nu)(b)$ represents the nonlinear terms which are functions of products of the first-order background metric and the second-order dynamic perturbation.

It was shown[4] that the source terms above which neglect nonlinearities, yield precisely the energy flux of the quadrupole formula. For free fall, attention must also be focused on the remaining nonlinear contributions to b'. The analysis of the contributions from the many source terms is very lengthy and difficult and will be fully delineated in another publication[6]. As anticipated[4], certain parts of the source for b' combine to give the dominant asymptotic contribution

$$b' = -4\frac{G^3m^3}{\alpha^2\rho_0^2}\quad\frac{(t-\rho)^2}{\rho}$$

where ρ is the radial coordinate. This yields a gravitational energy flux

$$\frac{\mathrm{d}E}{\mathrm{d}t} = -16\frac{G^5m^6}{\alpha^4\rho_0^4}(t-\rho)^2$$

during the early stages of free fall.

Unlike electrodynamics, the radiated energy is dependent upon the structure of the sources. This is not surprising because of the important role of nonlinearities which are absent in electrodynamics. Indeed, it was shown[4] that the linear terms alone imply a structure-independent radiation.

For a given separation and mass, the flux increases with diminishing size ρ_0. This is understandable because of the increased background field intensity, which contributes through the nonlinearities to the radiation. It is to be emphasised that the expression for the flux loses its validity if ρ_0 is allowed to become so small as to violate the weak-field condition.

The harmonic coordinate condition, which has caused others difficulty for asymptotic calculations, has not been employed. Moreover, the problem of avoiding the intrusion of incoming radiation is solved by the development from an initially static configuration.

The usual derivation of the quadrupole formula treats the retarded integrals as for compact sources in electrodynamics. Moreover, various surface integrals containing the energy–momentum pseudotensor are discarded on application of the Gauss theorem. These procedures are justified both in electrodynamics for compact sources and in general relativity when the compact sources dominate the field sources. During free fall, however, the field sources are of the same order as the compact sources and the analysis leading to the quadrupole formula is inapplicable.

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Stable photoelectrochemical cells for the splitting of water

FOLLOWING Fujishima and Honda's suggestion of water photolysis by a photoelectrochemical cell[1], no stable cell has been reported (but compare refs 2, 3) capable of water decomposition without the application of either an external bias or a pH gradient. The main problems have been to find stable anodes and cathodes with sufficiently negative and positive critical photopotentials, respectively, so that they may be suitably combined in a self-driving photo cell producing hydrogen and oxygen from water. We report here that the anodes n-TiO_2 and n-$SrTiO_3$ and the cathodes p-CdTe and p-GaP can be combined to form four separate stable self-driven cells: n-TiO_2–p-CdTe, n-TiO_2–p-GaP, n-$SrTiO_3$–p-CdTe and n-$SrTiO_3$–p-GaP capable of water photoelectrolysis.

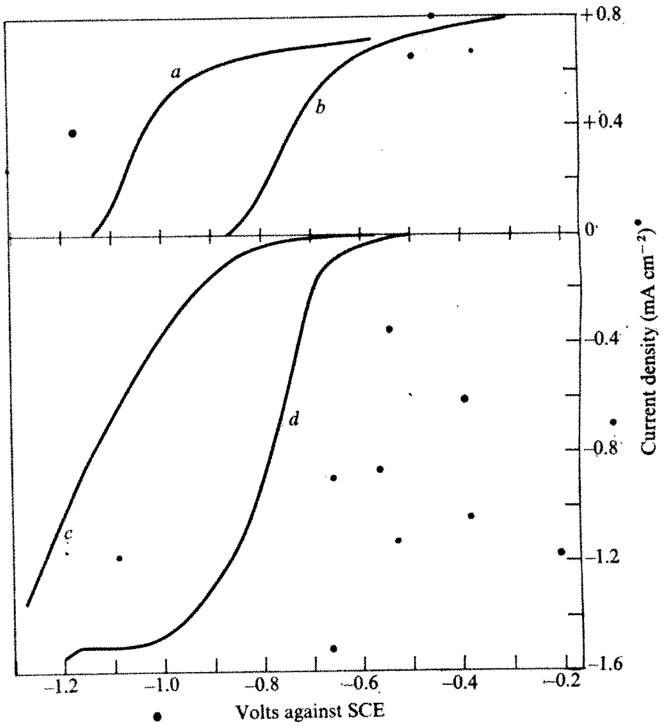
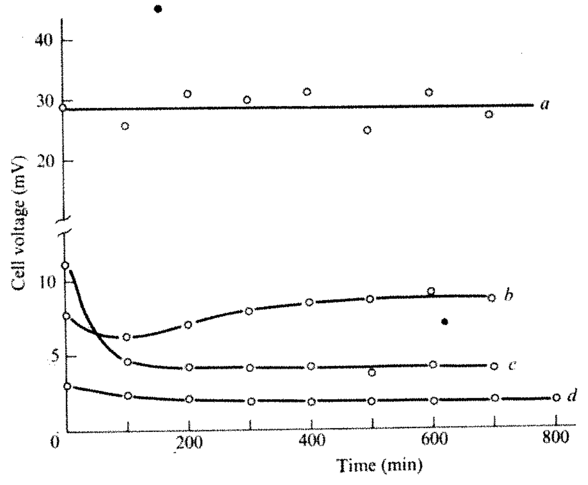


Fig. 1 Photocurrent–potential curves of semiconductor electrodes against Pt in 1 M NaOH, 25 °C. *a*, n-$SrTiO_3$; *b*, n-TiO_2; *c*, p-CdTe; *d*, p-GaP. Potential sweep rate: 10 mV s^{-1}. Areas: n-TiO_2, p-CdTe and p-GaP, 0.0491 cm^2, n-$SrTiO_3$, 0.0594 cm^2, 0.05 W cm^{-2}.

TiO_2 (001 face) and $SrTiO_3$ polished single crystals were reduced in H_2 at 800 and 900 °C for 5 and 3 h, respectively, to form low-resistance n-type crystals. Ohmic contacts to both

Fig. 2 Cell voltage–time curves of self-driven electrochemical hydrogen producers. *a*, $SrTiO_3$–GaP; *b*, $SrTiO_3$–CdTe; *c*, TiO_2–GaP; *d*, TiO_2–CdTe. External resistance 1 kΩ. Areas: n-TiO_2, p-CdTe and p-GaP, 0.0491 cm^2, n-$SrTiO_3$, 0.0594 cm^2 in 1 M NaOH, light intensity 0.05 W cm^{-2}, 25 °C.



crystals were obtained by soldering indium on to the crystals. An ohmic contact to a p-CdTe (undoped; 110 face) was formed by depositing gold on a silver diffused layer. An ohmic contact to p-GaP (Zn doped; 100 face) was formed by soldering an In–Zn alloy on to the back of the crystal. The crystals were positioned in Teflon holders and fixed with epoxy resin.

Each pair of electrodes was positioned in adjacent compartments of an electrochemical cell with a water jacket. The light from a 900-W xenon lamp was split equally with front surface mirrors of evaporated aluminium film on glass plates and used to irradiate each electrode.

The photocurrent–potential curves of the four electrodes in 1 M NaOH, light intensity 0.05 W cm^{-2} at 25 °C are shown in Fig. 1. The two n-type electrodes n-TiO_2 and n-$SrTiO_3$ can be combined with both p-CdTe and p-GaP to form self-driving photoelectrolysis cells.

The stabilities of the cells were determined by measuring the cell voltage as a function of time with an external resistance of 1 kΩ (Fig. 2). The cell voltage in each case after reaching a steady-state value after some 3 h was still stable after 12 h, although there were some fluctuations due to instability in the light source. Some electrodes were used satisfactorily for a total of 50 h in various cells.

The chemical output power of each cell (Fig. 3) was calculated from the maximum cathodic current densities based on the free energy change of $H_{2(g)}$ to $H_2O_{(l)}$, that is, 1.23 (V) × i, where i is the cathodic current density.

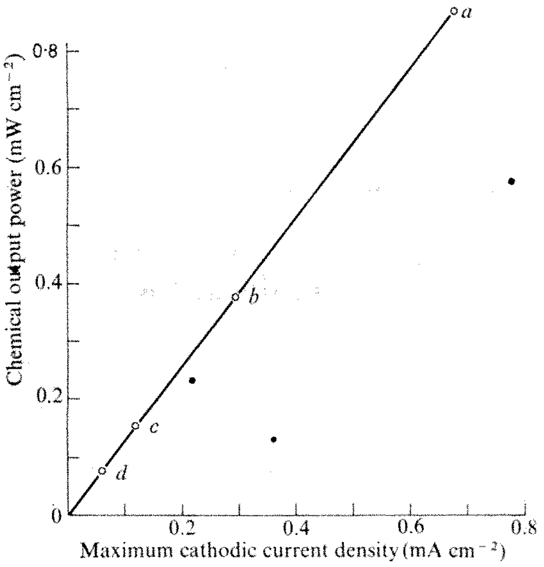


Fig. 3 Maximum cathodic current densities of self-driven electrochemical hydrogen producers and their chemical output power densities based on the free energy change of $H_{2(g)}$ to $H_2O_{(l)}$, that is expressed as 1.23(V) × i, where i is the current density. a, $SrTiO_3$–GaP; b, $SrTiO_3$–CdTe; c, TiO_2–GaP; d, TiO_2–CdTe. Areas: n-TiO_2, p-CdTe and p-GaP, 0.0491 cm^2, n-$SrTiO_3$, 0.0594 cm^2. 1 M NaOH, light intensity 0.05 W cm^{-2}, 25 °C.

The efficiencies of the cells in xenon light were calculated and are listed in Table 1. The efficiencies are still too low to be of practical applicability, but the difficulty is caused by the large energy gaps of the photo-anodes n-TiO_2 (3.0 eV) and n-$SrTiO_3$ (3.8 eV). From the energy gaps of p-GaP (2.25 eV) and p-CdTe (1.5 eV), it is possible to estimate that these electrodes can use up to 2 and 13% of the solar spectrum, respectively.

Thus, the major problem if this method is to be considered as a contender for a method of H_2 production is to find a stable anode (E_G 1.0–1.8 eV) with a critical photopotential such that it can be efficiently combined with p-CdTe in a self-driving hydrogen producer. The present results do not preclude the development of cells with an efficiency of > 7%.

Table 1 Efficiencies of cells in xenon light*

Cell†	Efficiency in xenon light (%)
n-TiO_2–p–CdTe	0.044
n-TiO_2–p-GaP	0.098
n-$SrTiO_3$–p-CdTe	0.18
n-$SrTiO_3$–p-GaP	0.67

*Conditions: 1 M NaOH, 25 °C, light intensity, 0.05 W cm^{-2}.
†Electrode areas: n-TiO_2, p-CdTe, p-GaP, 0.0491 cm^2; n-$SrTiO_3$, 0.0594 cm^2.

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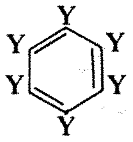
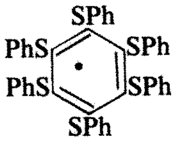
Crystal and molecular structure of a 'hexa-host' inclusion compound

WE recently suggested[1], and found experimental support for[1,2], a totally new concept for the design of inclusion compounds. Using this design concept we prepared a series of host compounds based on a hexa-substituted benzene ring (I), and we now report the results of the first X-ray structural study on one of these compounds.



(I)



(II)

The carbon tetrachloride adduct of hexa-phenylthiobenzene (II) is trigonal with a unit cell (a=14.263; c=20.717 Å) containing three molecules of $C_{42}H_{30}S_6$. A host–guest (carbon tetrachloride) ratio of 1:2 was found by microanalysis for chlorine. The space group is either R3 or R$\bar{3}$ (ref. 3), the centrosymmetric space group (R$\bar{3}$) was chosen and this choice has been justified by the success of the subsequent analysis. The structure was solved by direct methods using 2071 independent reflections measured with Mo–Kα radiation on a Hilger–Watts automatic diffractometer (using a Lindemann capillary for crystal containment), and refined to a final R value of 0.055. All the hydrogen atoms have been located and allowed for, as have the guest molecules. Full details of the structural analysis will be published elsewhere.

Figure 1 shows a general view of the host molecule. It is centred on a point of $\bar{3}$ symmetry; thus the crystallographically equivalent 'legs' of the molecule point alternately above and

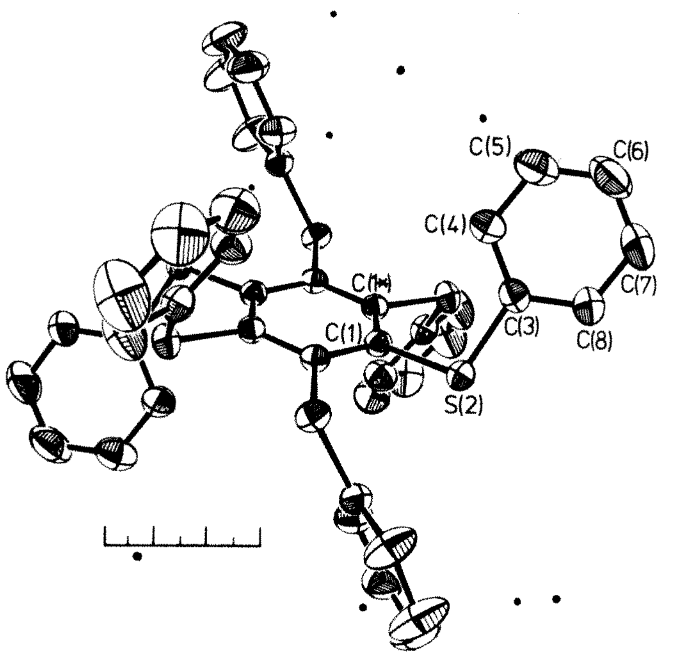
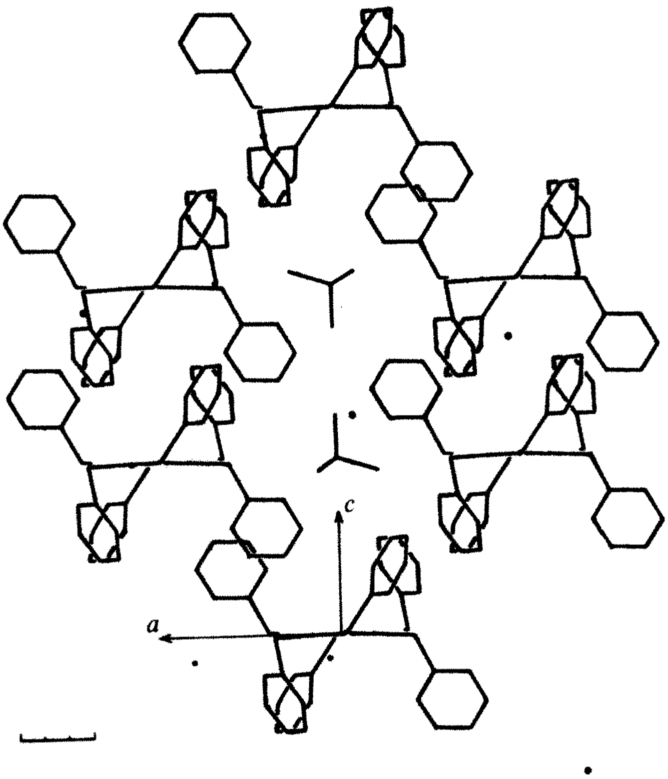


Fig. 1 An ORTEP drawing showing a general view of the molecular structure of host molecule (II) in the crystal of the CCl_4 clathrate. (The hydrogen atoms are not shown). Bar represents 3 Å.

below the plane of the central benzene ring. The relative orientation of the central and side-chain aromatic rings may be appreciated by a consideration of the torsion angles C(1*)–C(1)–S(2)–C(3) and C(1)–S(2)–C(3)–C(4), which are 56° and 28° respectively.

Figure 2 illustrates the host–guest packing arrangement in the crystal; the two host molecules which complete the cage (which lie above and below the cavity as reviewed in the direction shown) have been omitted for clarity. The two CCl_4 guest molecules in the clathrate cage are orientated such that a C–Cl bond of each is co-linear with the *c* axis. Although relatively small gaps have been detected in the cage wall these are insufficiently large to allow the guest molecules to escape unhindered. Solvent loss does in fact occur slowly when the crystals are left in air at room temperature, with apparent complete disruption of the crystal lattice.

Fig. 2 An illustration of the host–guest packing in the crystal of the CCl_4 clathrate of (II), as viewed on to the *ac* plane. Two host molecules which lie above and below the cavity as viewed in this direction have been excluded to show the guest molecules more clearly. Bar represents 3 Å.



The crystal packing forces seem to be of the normal van der Waals' type, no evidence having been found to suggest marked charge transfer interactions between host and guest molecules.

A host–guest ratio of 1:2 has also been found for the guests CCl_3CH_3 and CCl_3SCl, though guest species CCl_3Br and CCl_3NO_2 form complexes with a 1:1 ratio. Recrystallisation of (II) from the solvent CCl_3CCl_2H gives unsolvated triclinic crystals.

Further studies on other 'hexa-host' molecules indicate that trigonal symmetry is not always encountered in the inclusion compounds formed. A study of the complex of hexakis (benzylthiomethyl) benzene (formula (I), Y=CH_2SCH_2Ph) with dioxane has shown the crystals to be monoclinic, space group $P2_1/c$, with two host and two guest molecules in the unit cell.

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Effect of magnetic field on reduction of iron oxides: magnetite and wüstite

A STRONG magnetic field (500–1,400 Oe) was reported by Skorski[1] to cause an increase in the reduction rate of haematite (Fe_2O_3) to metallic iron when H_2 was used as the reducing agent. This effect was attributed to the magnetic properties of the H_2, because neither CH_4 nor CO produced a similar increase when under the influence of a strong magnetic field. Although Svare[2] disputed this explanation, Peters[3] offered an alternative one, based on thermodynamics, which indicated that an increase in reaction rate is expected under the influence of a strong magnetic field when the reactants were relatively non-magnetic and the products were strongly magnetic—as with reduction of haematite to iron. We report here our results of studying magnetite and wüstite reduction to iron, which show an increased reduction rate for magnetite, under the influence of a strong magnetic field, but an inexplicably decreased rate for the wüstite reduction.

Confirmation of the effect noted by Skorski[1] was attempted by Rowe *et al.*[4]. To avoid the complication arising from the two-step process of haematite to iron reduction with H_2 below 550 °C ($Fe_2O_3 \rightarrow Fe_3O_4 \rightarrow Fe$), Rowe *et al.* examined the simpler nickel oxide–nickel system. The NiO → Ni reduction meets Peters' criterion for expected reduction rate effects from applied magnetic fields almost as well as the $Fe_2O_3 \rightarrow Fe_3O_4 \rightarrow Fe$ system does. That is, NiO is relatively non-magnetic (paramagnetic) whereas Ni is ferromagnetic (saturation magnetisation $\simeq$ 54). Fe_2O_3 is relatively non-magnetic (antiferromagnetic), while metallic Fe is also ferromagnetic (saturation magnetisation $\simeq$ 218). (Fe_3O_4 is also relatively magnetic, being ferromagnetic with saturation magnetisation $\simeq$ 92.) Rowe *et al.* found that application of a strong magnetic field (~4,200 Oe) produced no detectable difference in the reduction rate of NiO to metallic Ni and therefore failed to confirm the general effect expected in the reduction of a non-magnetic species to a magnetic one.

Having observed no difference in reduction rate of NiO to Ni on the application of a strong magnetic field, we now check more directly Skorski's observation on Fe_2O_3 reduction. At temperatures less than 550 °C, Fe_3O_4 is formed as an intermediate; Skorski did not attempt to account for this effect. To eliminate this complication, we decided to investigate here the reduction of magnetite and wüstite (FeO) to metallic iron. According to Peters' thermodynamic argument, the rate of reduction of FeO to Fe should be enhanced to a greater degree than expected for Fe_3O_4 to Fe, since magnetite is more strongly magnetic than wüstite. That is, the change in saturation magnetisation is greater in reducing FeO to Fe than in reducing Fe_3O_4 to Fe. Also, both of these reaction rates should be enhanced to a greater extent than in the NiO → Ni case, if Peters is correct.

Our apparatus[4], which allows continuous observation throughout the reduction (an advantage over Skorski's 'observation after the fact' method), consists of an electrobalance (sensitive to 10^{-6} g) connected to a strip chart recorder to trace the weight of reactant (Fe_3O_4 or FeO) plus product (Fe). When the strong magnetic field is applied, the apparent weight (saturation magnetisation) of the Fe_3O_4 and Fe or the FeO and Fe are recorded. The temperature was measured by means of a thermocouple inserted nearby the quartz sample pan.

Before each run, the electrobalance system (including sample) was flushed at room temperature with 20% H_2 in N_2 carrier at a rate of 65 ml min^{-1} for at least 1 h to assure a constant reaction atmosphere for each run. The gas flow was continued until the reaction was essentially 100% complete and the experiment was ended. We used ~1–3-mg samples of powdered Fe_3O_4 and FeO. The Fe_3O_4 samples were powdered to 200 mesh; finely powdered FeO samples were used but the mesh was not known. All experiments were begun at room temperature (~24 °C) and reached ~385 °C (for magnetite) after about 8 min, ~490 °C (for wüstite) after about 10 min. Reaction did not occur until near the reaction temperature.

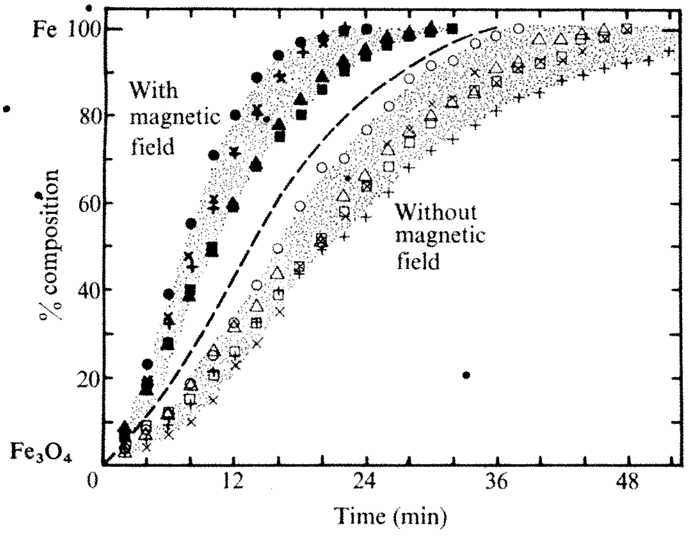

Fig. 1 Reduction of Fe_3O_4 to metallic iron with a 65-ml-min^{-1} flow of 20% H_2 in N_2 carrier at ~385 °C. Points were hand-read from a strip chart recording for construction of Fig. 1 so that data taken under the influence of a strong magnetic field can be directly compared with those without the strong magnetic field. The mass data were recorded continuously. Data on the left side of the dashed line in Fig. 1 were recorded after the insertion of an ~4,200-Oe permanent magnet. Initial sample weights (Fe_3O_4): ●, 0.98 mg; +, 0.98 mg; ▲, 1.29 mg; ■, 2.27 mg; ×, 1.42 mg. Data on the right side of the dashed line in Fig. 1 were recorded in the Earth's magnetic field (~0.5 Oe). Initial sample weights (Fe_3O_4): ○, 1.24 mg; +, 1.53 mg; △, 2.22 mg; □, 1.54 mg; ×, 1.18 mg.

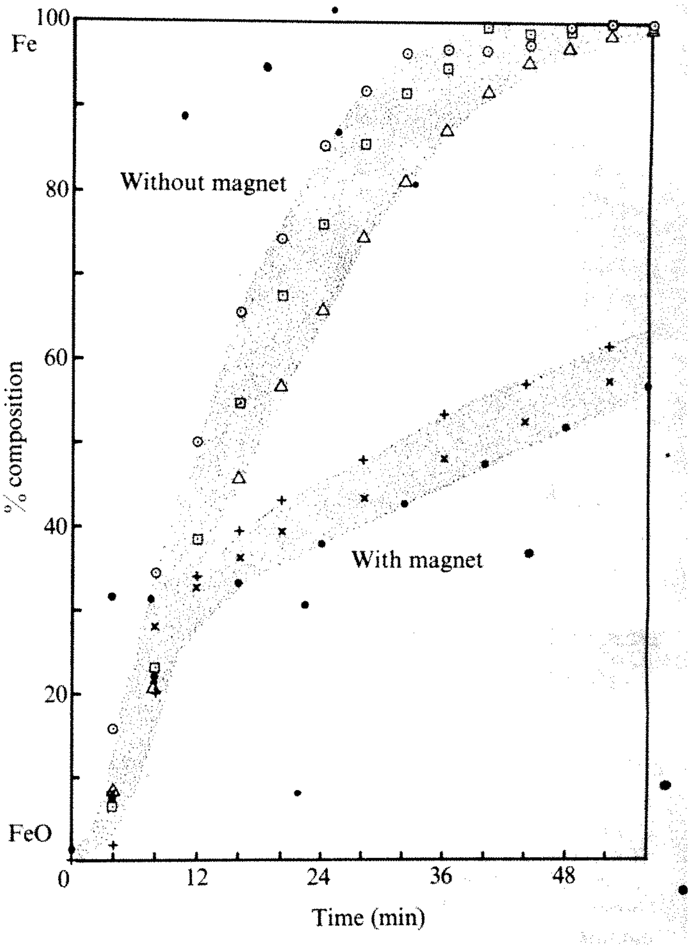

Fig. 2 Reduction of wüstite to metallic iron with a 65-ml min^{-1} flow of 20% H_2 in N_2 carrier at ~490 °C. Points were hand-read from a strip chart recording (see Fig. 1 legend). The mass data were recorded continuously. Data on the left side of Fig. 2 were recorded in the Earth's magnetic field (~0.5 Oe) those on the right side were recorded after the insertion of an ~4,200-Oe permanent magnet. Initial sample weights (FeO): ○, 1.31 mg; □, 1.76 mg; △, 2.51 mg; ●, 2.46 mg; +, 1.05 mg; ×, 2.25 mg.

For magnetite, five runs were performed in the Earth's field, and five after the insertion of an ~4,200-Oe permanent magnet (Fig. 1). The reductions were all carried to essential completion (as confirmed by stoichiometry), and the reduction rates compared. Skorski's[1] reductions were not generally carried to completion. Figure 1 shows that the reduction of magnetite to iron occurred at a significantly faster rate under the influence of the strong magnetic field, thus confirming Skorski's observation by an independent technique. This is not, however, direct confirmation because we found the reaction rate was increased on reducing Fe_3O_4 to Fe, whereas Skorski started with Fe_2O_3 and ended with an Fe_2O_3, Fe_3O_4 and Fe mixture of unknown composition.

For the wüstite reduction, three runs were conducted with a strong magnet, and three without (Fig. 2). The reductions were all carried to (and recorded to) essential completion, as for magnetite, and the rates compared. Although the data for the wüstite reduction in the presence of the strong magnetic field are not shown to completion in Fig. 2, the difference in rates brought about by the magnetic field is apparent.

Surprisingly, we found that the application of a strong magnetic field during the wüstite reduction with H_2 resulted in a significantly slower reaction rate than when the reduction was conducted in the Earth's field. This supports our earlier suspicion that Peters's[3] thermodynamic argument was not the determining factor in the reductions studied to date (refs 1, 4 and present work). In all cases, the general requirement is met that the product be more highly magnetic than the reactant, but a strong magnetic field affects the reduction rates as follows:

$NiO \rightarrow Ni$	no observable effect
$Fe_2O_3 \rightarrow Fe_3O_4 \rightarrow Fe$ $Fe_3O_4 \rightarrow Fe$	reduction is speeded up
$FeO \rightarrow Fe$	reduction is slowed down

We are not able to explain these data at present. This work was supported by NASA.

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Computer simulation and statistical geometric model for contacts in binary random two-dimensional disk packings

Experimental investigation of the characteristics of multicomponent packings of particles would involve a large number of manipulations for even very few components in the mixture, and each additional component would increase this number enormously. A method of calculating the properties of such mixtures would be of interest if only as a framework within which to design experiments. One difficulty is that for this very reason a theoretical approach cannot be subjected to a thorough experimental verification. We therefore think it useful to take the problem further by comparing two previously published theoretical approaches, by a statistical geometric model[1] and a computer simulation[2], for calculating the contacts between disks in two-dimensional multicomponent random mixtures.

The average total coordination number in a packing is defined as the total number of contacts in the packing divided by the total number of disks in the packing (C). In multicomponent packings this may be further subdivided: first, into the individual average coordination number for each disk size (c_i), that is, the total number of contacts on disks of a given size divided by the number of disks of that size; second, into the average coordination number for each size by each of the other disk sizes in the packing (p_{ij}).

In a binary packing, these various coordination numbers are related by the following expressions:

$$C = (n_1c_1+n_2c_2)/(n_1+n_2) \tag{1}$$

$$c_1 = p_{11}+p_{12} \tag{2a}$$

$$c_2 = p_{21}+p_{22} \tag{2b}$$

Note that $p_{12} \neq p_{21}$ but $n_1p_{12} = n_2p_{21}$

It was shown previously[1] that the average coordination number on each disk size (c_i) could be calculated from a disk size distribution (relative numbers of disks n_i and their diameters d_i) using a simple statistical geometric model of an idealised gapless packing of which the space was divided up into triangular subunits. The matrix of (p_{ij}) values can also be calculated from the model through the relative numbers of triangular subunits (T_{ijk}) calculated by expressions (3) and (4) in ref. 1. Each of these subunits includes three contacts and the relative numbers of the different types of contact between disks can therefore be calculated. For a binary mixture there are three types of contact and expressing these as fractions of the total number of contacts we have

$$t_{11} = (T_{111}\times 3+T_{112})/3 \tag{3a}$$

$$t_{22} = (T_{222}\times 3+T_{122})/3 \tag{3b}$$

$$t_{12} = t_{21} = (T_{112}\times 2+T_{122}\times 2)/3 \tag{3c}$$

$$t_{11}+t_{22}+t_{21} = 1 \tag{4}$$

By definition and for a binary mixture

t_{11} = number of type 11 contacts/total number of contacts

$$t_{11} = (n_1\,p_{11})/(n_1c_1+n_2c_2) \tag{5a}$$

$$t_{22} = (n_2\,p_{22})/(n_1c_1+n_2c_2) \tag{5b}$$

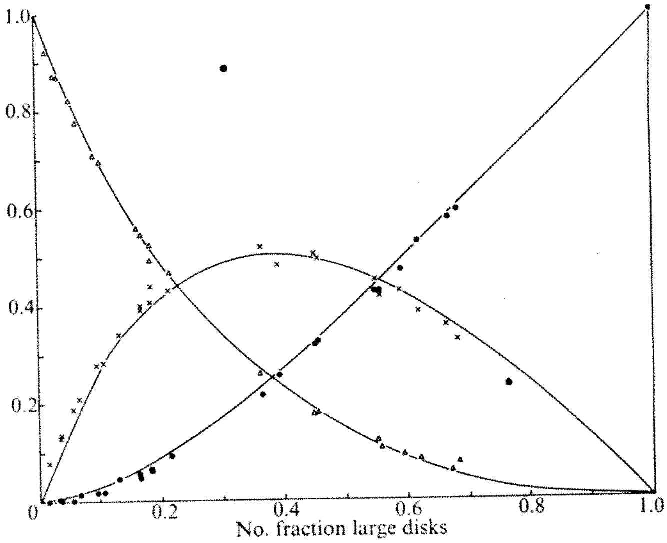
$$t_{21} = t_{12} = (n_1p_{12}+n_2p_{21})/(n_1c_1+n_2c_2) \tag{5c}$$

By elimination between expressions (2), (3) and (5) we may calculate values of p_{ij}.

As reported in ref. 2, the process simulated is that of disks being put into a two-dimensional box in random order from a random point and allowed to fall and roll until they attain a dynamically stable position. No adhesion or rebound is considered and once in position a disk is assumed not to move. The various coordination numbers are obtained by counting after rejection of the disks at the edge.

It is well known[3] that the porosity and coordination number in packings depend not only on geometrical factors (size distribution) but also on what may be called mechanical factors such as the conditions in which the packing is formed: vibrated, settled in water dropped from a height, or the properties of the grains other than size; their density, surface roughness, existence of attractive or repulsive forces. For example, a packing of monosize glass spheres can have a porosity of 0.40 or 0.36 and an average coordination number of 8 or 9 depending on whether the packing is vibrated or not. Grains with a higher density or with attractive forces between them tend to pack to lower porosities than grains with surface roughness or repulsive forces between them. As an example of these effects it is possible to simulate a looser packing with a lower total coordination number by including an adhesional probability in the computer simulation. When a disk is put into the box and touches another disk a random number is compared with the assumed adhesional probability: if the random number is smaller than the adhesional probability the disk is fixed in that position, if it is greater the disk continues to roll until the next contact when the procedure is repeated or the disk reaches a stable position. All the results reported here are

Fig. 1 Comparison of coordination points from the model with computer simulation. Diameter ratio 2.5. Values of t_{ij} are shown as curves, and computer simulation results are shown as points: △, t_{11}; ×, t_{12}; ●, t_{22}.



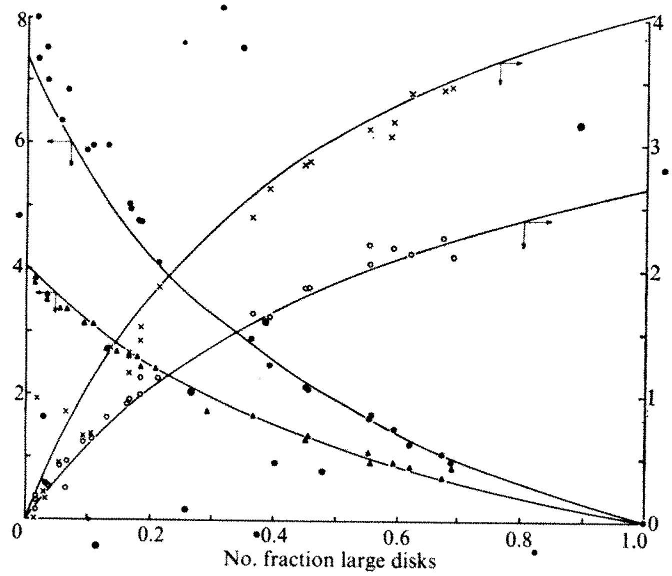


Fig. 2 Comparison of coordination points from the model with the computer simulation. Diameter ratio 2.5. Values of $_{ij}$ from the model multiplied by the compaction factor are shown as curves, and computer simulation results are shown as points: ▲, p_{11}; ○, p_{12}; ●, p_{21}; ×, p_{22}.

for an adhesional probability of 0%. Here the model is concerned only with geometrical factors and relates to an idealised compact gapless packing, with an average total coordination number of 6. The computer simulation includes mechanical factors to a certain extent and gives values for a more realistic packing with a constant average total coordination number of 4.02±0.04 for all mixtures considered here. This geometrical invariant may be used to define a 'compaction factor' which represents the degree to which mechanical factors allow real packings (here computer simulated) to approach the geometry of the idealised packing. In this case the factor is 4.02/6.00 = 0.67. Multiplying by this factor allows the different coordination numbers obtained from the model to be compared with the computer simulation. The figures show this comparison. Figure 1 (analogous to Fig. 3 in ref. 1) shows values of t_{ij} from the model as smooth curves and results from the computer simulation as points; the agreement is excellent. Figure 2 (analogous to Fig. 6 in ref. 2) gives values of p_{ij} from the model multiplied by the compaction factor as smooth curves and the results from the computer simulation as points. The agreement is excellent which indicates the concept of the compaction factor.

The comparison between the coordination numbers for two-dimensional binary random disk packings as calculated by computer simulation and a theoretical model shows a good agreement between the two methods. Notably the invariability of the average total coordination number with size ratios and mixture proportions is confirmed. This allows the definition of a 'compaction factor' which allows the results of the theoretical model for an idealised gapless packing to be used to calculate coordination numbers for real packings.

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Gravity in Zambia

A RECONNAISSANCE gravity survey of the Republic of Zambia was carried out from 1971 to 1975 by the Geological Survey of Zambia in collaboration with the University of Zambia and The University of Michigan[1]. We present here a Bouguer anomaly map and preliminary analysis of the gravity data, in the context of the principal tectonic elements and structural characteristics of eastern and southern Africa.

Zambia is situated on the Central African Plateau which slopes gently from an elevation of 1,000 m in the south and west of Zambia to a maximum elevation of >2,000 m in the northeast. The characteristic gently sloping terrain is broken in the extreme north by the Tanganyika and Mweru rifts and in the east and south, respectively, by the Luangwa and mid-Zambezi rifts, which contain Karroo (Permo-Triassic) sediments and volcanic rocks. The plateau comprises principally Pan-African (450–750 Myr) and older igneous and metamorphic basement rocks. In the west, the basement is in places broken by fault block basins, but the distribution of these is obscured by a blanket of Quaternary Kalahari sands.

Over 2,700 gravity stations were established along the primary and secondary road network of Zambia, at approximately 10–15-km intervals, with a characteristic density of one station per 400 km². All stations were referenced to the previously established East African primary net[2]. Free-air and simple Bouguer anomalies were calculated using the 1930 International Gravity Formula and a density of 2.67 g cm⁻³. Elevations for about one-third of the stations were taken from topographic map contours; at the remaining stations, elevations were determined with aneroid barometers. A mean uncertainty of ±2 mgal, arising principally from uncertainties in elevation, is typical.

Our Bouguer anomaly map of Zambia (Fig. 1) shows Bouguer anomalies ranging between −200 and −75 mgal, with a mean near −140 mgal. The regions in the north and northwest marked by the −160-mgal contour lie along the axis of the so-called Great Negative Bouguer Gravity Anomaly[3] of Africa; the long and continuous −120-mgal contour in the south marks its approximate southern boundary. The overall grain of the map is dominantly northeast–southwest, a tectonic trend which is prominently displayed by the Upemba graben in southern Zaire, the Mweru and Luangwa rifts, the middle Zambezi valley, and which continues to the south-west into northern Botswana[4].

Some of the individual features of the map can be clearly identified with structural elements, the best example being the elongated gravity trough in eastern Zambia which coincides closely with the Luangwa rift. The southern terminus of the Tanganyika rift, and the northern margin of the Zambezi rift along Lake Kariba also show gravity depressions. The arcuate trend of the −140-mgal contour in the northern part of central and western Zambia follows closely the curvature of the Lufilian arc, a Pan-African age structural element that hosts the rich copper mineralisation of Zambia and southern Zaire. The moderate gravity depression in central western Zambia is associated with a zone of persistent seismic activity[5]; the large nearly circular depression in northern Zambia lies within a region of Precambrian platform sediments, and could represent a small sedimentary basin.

Three regions show prominent gravity highs: the eastern border with Malawi, the region south of Lake Mweru, and the region north of Lake Kariba. In each of these areas the anomalies reach −80 mgal with gravity relief of at least 40 mgal. These highs are not associated with any obvious structural elements or topographic settings; they are in areas with elevations of 900–1,800 m.

Residual anomalies were obtained by subtracting a smooth regional anomaly field from the observed Bouguer anomaly field. The regional pattern was estimated by a number of techniques, including free-hand smoothing, Fourier and polynomial surface-fitting methods, and a mean anomaly against elevation parameterisation. Each representation of the regional field yielded different residuals, but some anomalous regions were common to all of the residual fields. Those regions, each with residual anomalies >20 mgal (inset map of Fig. 1) display a north-east–south-west grain, as does the anomaly map. The most prominent feature is the chain of positive residuals extending from south-western Zambia north-eastward along the southern margin of the country. This remarkable feature merely traverses Zambia; it can be traced south-westward through Botswana to the Namibia border[4], and continues north-eastward into the Malawi highlands[6], approaching 2,000 km in length. This prominent positive residual buttresses the southern margin of the Great Negative Anomaly throughout central Africa.

The negative residual over the Luangwa rift arises from the low-density Karroo sedimentary fill. A similar residual probably exists in the mid-Zambezi valley beneath Lake Kariba, but it has not been confirmed due to a lack of data from the valley and beyond in Rhodesia. The negative residual which extends from the north across the Zaire pedicle into the Zambian Copperbelt possibly reflects relatively thick sedimentary accumulations in the north, but is associated with the Copperbelt granites at its south-western terminus.

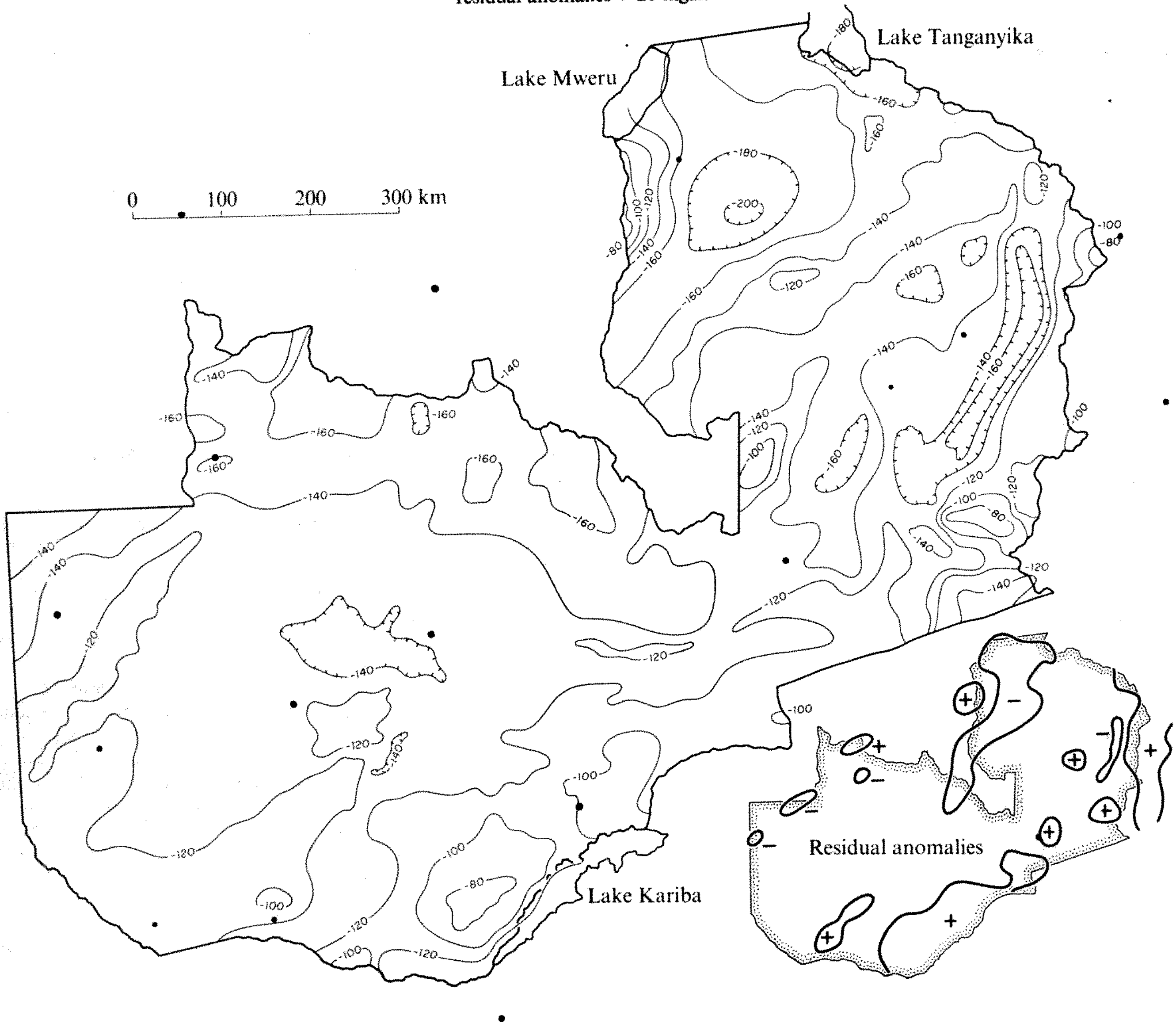
A scatter plot of Bouguer anomalies against elevation is shown in Fig. 2*a*. Stations at <800-m elevation lie exclusively within the Luangwa, Zambezi and Tanganyika troughs, and thus afford an opportunity to compare gravity variations in rift and non-rift settings. Regression lines were fitted to mean Bouguer anomalies and mean elevations in each 100-m elevation interval; these lines are shown in Fig. 2 and their parameters in Table 1, with similar, earlier analyses from east Africa[7,8].

The Zambia and Kenya regression lines have similar slopes in their respective rift and non-rift settings, but the Zambian intercepts in both settings are substantially more negative. The Zambian line for non-rift stations is almost the same as that determined by Woollard for 3°×3° mean anomalies in east Africa.

The different slopes in rift and non-rift areas implies different densities in the two settings, and suggests contrasting crustal and upper mantle structures. The significance of the different intercepts is unclear, but the differences may stem from the fact that in Woollard's[8] and our analyses, the lines were fitted to mean anomalies, while Khan and Mansfield[7] presented a subset of their individual station data, which may be biased away from the regional mean.

Free-air anomalies are also of interest because they can give to a first order an estimate of how closely a region approaches isostatic equilibrium. When isostasy prevails, free-air anomalies are usually less than a few tens of mgal, distributed randomly about zero, and show very little dependence on elevation. Woollard[8] gives an empirical relationship between free-air anomalies and elevation in isostatic regions as $FA = 0.0075h-6$, where FA is the free-air anomaly in mgal and h is the elevation in m.

Fig. 1 Bouguer gravity anomaly map of Zambia. Contour interval 20 mgal. Reduction density 2.67 g cm^{-3}. Inset map shows residual anomalies >20 mgal.



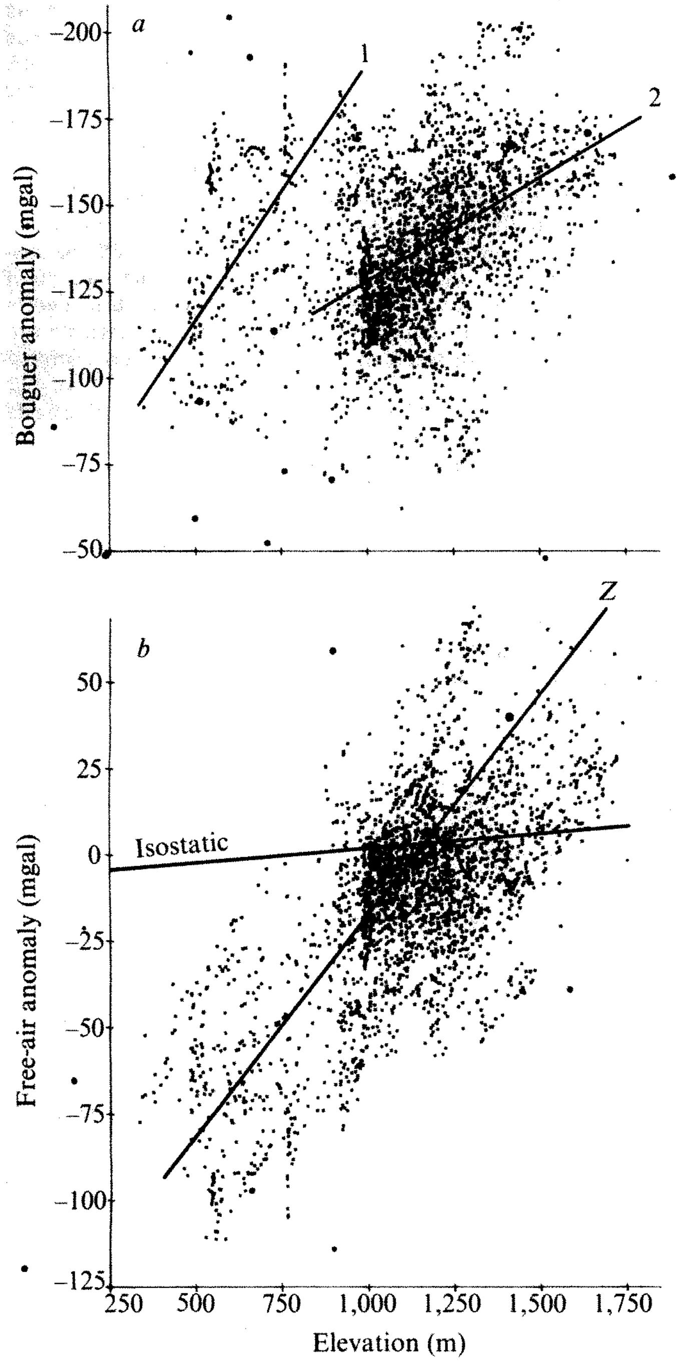


Fig. 2 *a*, Scatter plot of Bouguer anomalies against elevation, with regression lines for rift (1) and non-rift (2) stations. *b*, Scatter plot of free air anomalies against elevation. Line labelled Z represents Zambian data; line labelled 'Isostatic' represents empirical relationship of Woollard[8]. Both lines are fitted to mean anomalies and elevations within 100-m intervals.

Figure 2*b* is a scatter plot of free-air anomalies against elevation for Zambia, and shows a clear departure from the isostatic relationship and an obvious dependence of anomalies on elevation. Such a dependence of free-air anomalies on elevation is not uncommon in regions of considerable relief, but in Zambia, apart from the escarpments adjacent to the rifts, the terrain is very gentle.

It is, therefore, possible that the Central African Plateau may not be in isostatic equilibrium. Woollard[8] notes that a plateau undergoing uplift can exhibit a regional departure from isostasy. Considerable evidence is accumulating that suggests that this part of central Africa is dynamic and responding to tectonic stress. Widely distributed earthquake epicentres[5], tensional focal mechanisms[9,10], anomalous heat flow[11] and disrupted drainage as shown on satellite photography together make an interesting if not compelling case for contemporary tectonism (incipient rifting) in the Central African Plateau. In such a context, it seems possible that isostasy is yet to be fully established in this current phase of tectonism. If tensional block faulting continues, however, and becomes more widespread in the Central African Plateau, a restoration of isostatic equilibrium will probably follow.

Table 1 Regressions of Bouguer anomalies (mgal) on elevation (h, m)

	Zambia	Kenya[7]	East Africa[8] (3°×3° mean anomalies)
Rift	$-0.15h-41$	$-0.137h-4.31$	
Non-rift	$-0.06h-67$	$-0.082h-5.85$	$-0.063h-57$

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Electrical conductivity and tectonics of Scotland

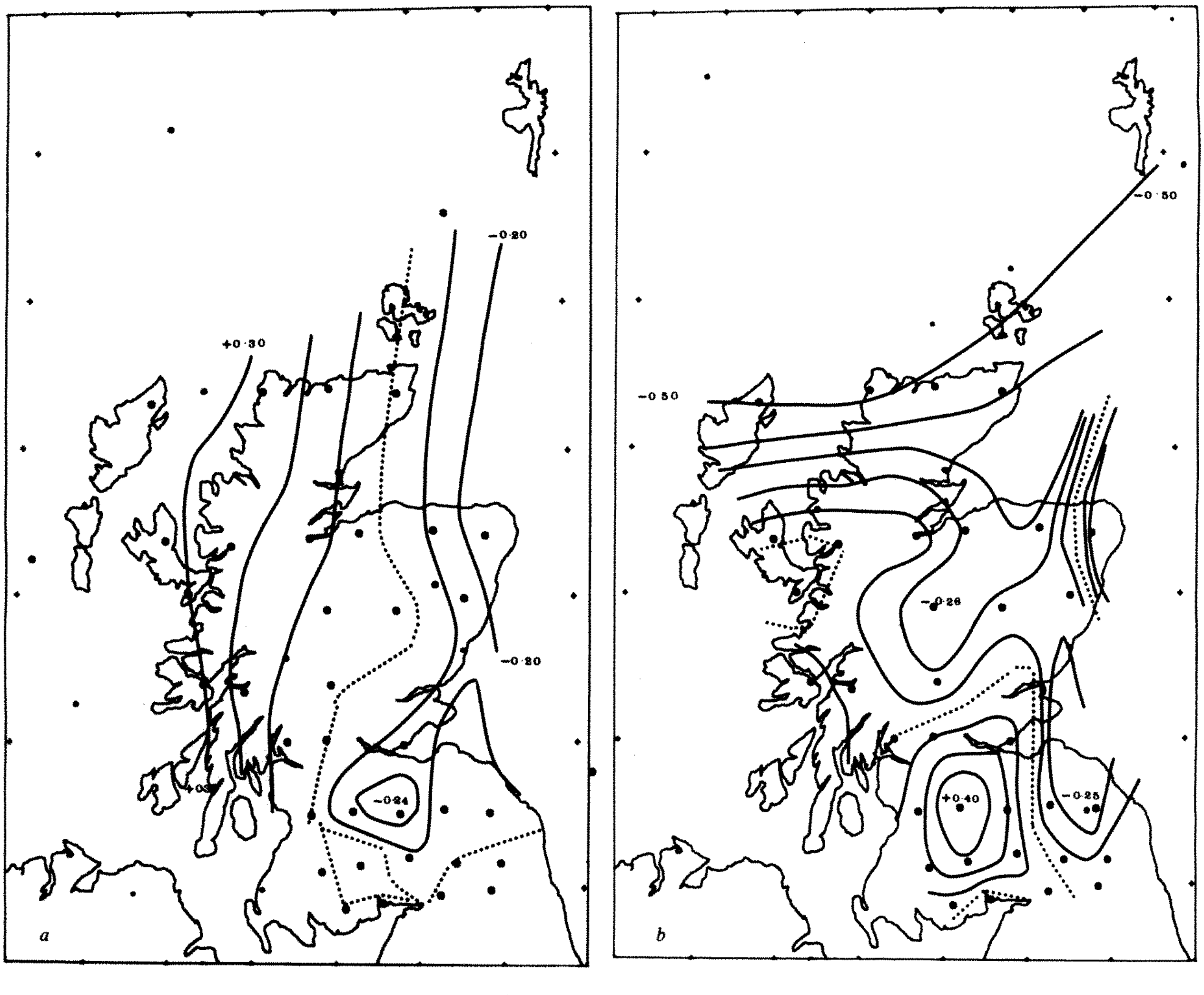
A REGION of anomalously high electrical conductivity in southern Scotland, known as the Eskdalemuir anomaly, has been discovered and mapped, in terms of related anomalies in time-varying geomagnetic disturbance fields[1,2]. Magnetotelluric soundings[3] have indicated the depth of the conductor as 12 km, at which depth Jacob[4] has reported an anomaly in seismic wave velocities. Bamford and Prodehl[5] have also found anomalous seismic wave velocities in this region. In 1973 the University of Edinburgh operated two magnetometer arrays which together covered nearly all of Scotland. In each array 20 three-component recording magnetometers of Gough–Reitzel type[6] were operated for 6–8 weeks. These arrays, together with extended magnetotelluric study of the Eskdalemuir anomaly[7], now make possible a fuller description and interpretation of conductive structures. Data from stations north of the Midland Valley, where only limited geomagnetic variation studies [8,9] had preceded ours, indicate further regions of anomalously high conductivity in Scotland. Full discussion and analysis of these data will be presented elsewhere. This note gives some preliminary indications of these conductivity anomalies and suggests possible tectonic implications.

Time-varying magnetic fields associated with induced currents in the solid earth, which may delineate conductive structures, are superposed on the source field due to currents in the magnetosphere and ionosphere. At the geomagnetic latitude of Scotland, source fields associated with polar electrojet or substorm activity have complicated morphologies, which may make the recognition of internal anomalies difficult. Induction in the Atlantic Ocean and the seas surrounding the British Isles provides an additional complication, particularly at the longer periods. Several techniques have been applied to the interpretation of the very large body of data from the arrays. These include the contouring of amplitudes and phases of Fourier transforms and the mapping of polarisation ellipses and of in-phase and quadrature-phase induction arrows. These procedures have been less successful in Scotland than elsewhere[10–13], presumably because of the superposition of spatially variable fields associated with the source and with the ocean on a complex geoelectric structure in northern Scotland.

One method which has proved useful in indicating the internal conductive structure is that of the hypothetical event[14], in which contoured maps of the vertical magnetic field component, Z, are found for a hypothetical uniform horizontal inducing field in any desired azimuth. If the vertical field can be represented by a linear combination of the horizontal fields, then the transfer functions $A(f)$ and $B(f)$, at the frequency f of the field can be calculated from $\bar{Z} = A(f)\,\bar{X} + B(f)\bar{Y} + \varepsilon(f)$. Here $\bar{X}$ and $\bar{Y}$ are the Fourier transforms at frequency f of the northward and eastward components of the field and $\varepsilon(f)$ is a small uncorrelated residual. By using appropriate values of $\bar{X}$ and $\bar{Y}$ to represent a desired hypothetical field and assuming that $\varepsilon(f)$ is negligible, one can obtain the Z response to the synthetic field using this equation and the estimated values of $A(f)$ and $B(f)$. The transfer functions are estimated from a number of variation events at each station[15]. While to some extent they reflect the relation of Z to X and Y in the source as well as in induced currents, the effect of the source on transfer functions derived from several events of differing source geometries and polarisations is smaller than that on a single event mapped, say, as Fourier transform coefficients. A second advantage of the hypothetical event method is that it substitutes for the natural source-fields, of complicated geometry and polarisation, a uniform, linearly polarised source field, the azimuth of which can be chosen to maximise the induced currents in a given structure. A third advantage is that this method facilitates unified interpretation of the results from the two consecutively-operated arrays.

Contoured maps of Z have been constructed for inducing fields linearly polarised in magnetic N, E, NW and NE azimuths, at periods in the range $300 \leq T \leq 5200$ s. For $T \geq 2400$ s the coastal and source field effects dominate the transfer functions in northern Scotland—as shown in Fig. 1(*a*) and (*b*)—but at shorter periods the internal currents predominate and produce a consistent and conspicuous pattern in the hypothetical-event Z contours in the range $300 \leq T \leq 1000$ s. Typical maps are shown in Fig. 2(*a*) and (*b*) for unit north-south inducing fields of periods 700 s and 1000 s. To a first approximation, a single current concentration produces closely spaced parallel contours in its vicinity. There may be a

Fig. 1 *a*, Z contours for a unit regional horizontal magnetic field directed eastwards. *b*, Z contours for a unit regional horizontal magnetic field directed northwards. In both cases, the values plotted are for the in-phase fields for a period $T = 2400$ s, the contour interval is 0.10 nT and the zero Z contours are represented by the dotted curves.



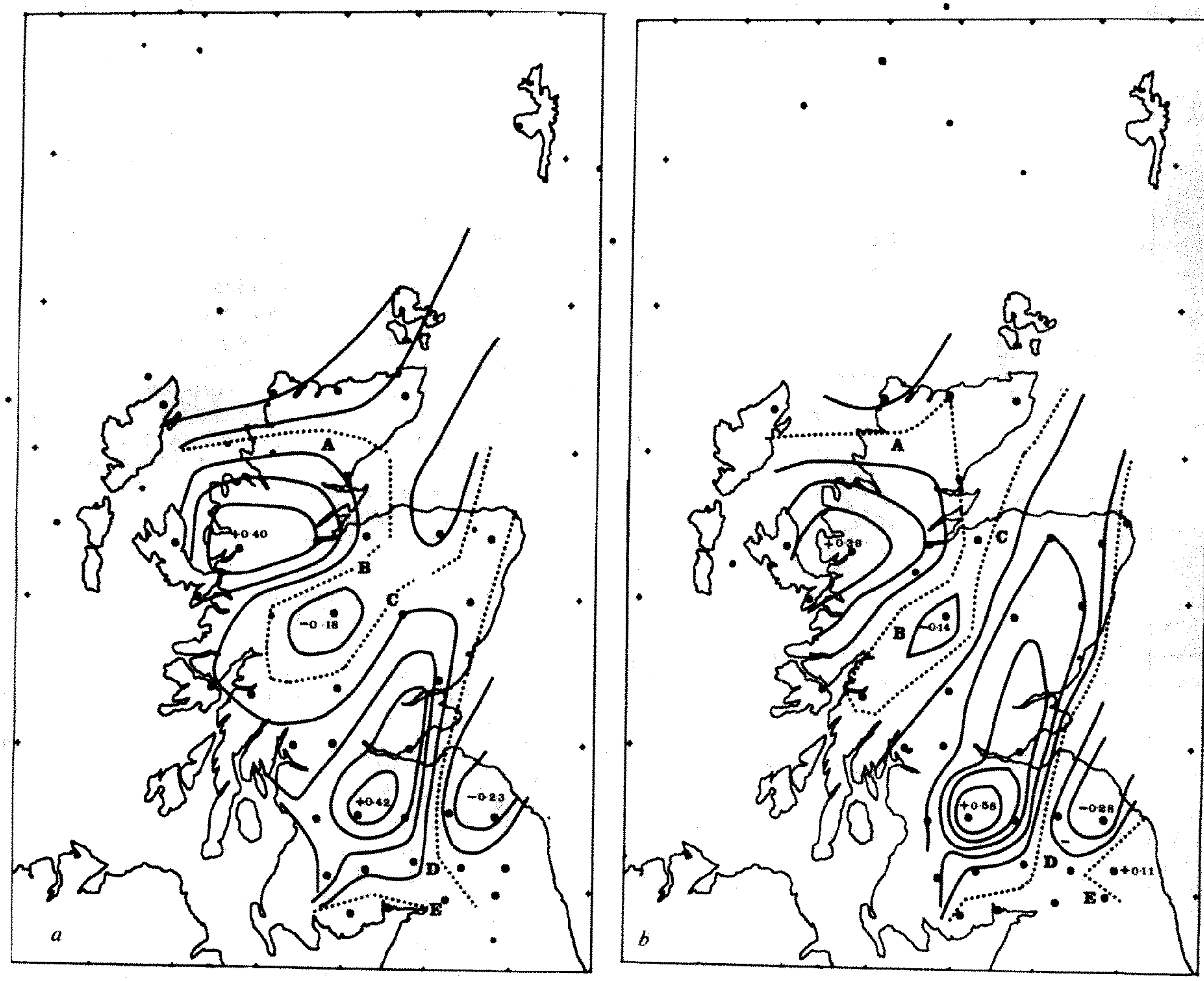


Fig. 2 Z contours for a unit regional horizontal field directed northwards. *a*, In-phase field for a period of 700 s. *b*, In-phase field for a period of 1000 s. The contour interval is 0.20 nT and the zero Z contours are represented by the dotted curves.

roughly central zero contour if there is no large regional gradient in Z due to other currents. In the maps the $Z = 0$ contours are delineated by dotted lines. For more complex current concentrations, the zero contours may not have the same significance. As shown by Bailey and Edwards[16], the Z field gradients are more useful for interpretation in this case. The Eskdalemuir anomaly, which may be associated with two regions of zero Z contours (D and E), is prominent in contour maps at all periods. Three other regions of zero Z contours appear consistently at the shorter periods. The first (*a*) is in the north-west corner of Sutherland, the second (*b*) runs along the Great Glen and the third (*c*) follows a path in eastern Scotland from the Moray Firth to Argyll. The widths of the Z anomalies set maximum limits of about 35 km on the depths of all these conductors, so they are within the crust. The significance of the Z contours is now being studied further and should lead to quantitative modelling of the conductive structures and correlation with other geological and geophysical features. For the present, attention is drawn to the interesting correlation between the three zero Z regions A, B and C (Fig. 2) and two Moinian and one Dalradian ophiolite suites regarded by Garson and Plant[17] as marking former subduction zones. These are indicated schematically in Fig. 3. These zones are associated with intense mineralisation–particularly the Dalradian zone which has, for example, well-defined outcrops of graphitic schists. The Moine belt of ophiolites, proposed by Garson and Plant as a late Precambrian subduction zone (750–800 Myr old), can be correlated in Sutherland with Z zero contour A and along the Great Glen with zero contour B. The third belt C seems to correlate with the Dalradian ophiolites, which Garson and Plant regard as marking a subduction zone active in Cambrian time. The three northern anomalous regions mapped by the magnetometer arrays may thus be related to these ancient subduction zones. It has been suggested that hydrated minerals in ophiolites may be highly conductive[18] but in ancient subduction zones other sources of high electrical conductivity probably exist as suggested by Camfield and Gough[19] for their proposed Proterozoic plate boundary in North America. Garson and Plant have also proposed two younger ophiolite suites along the Highland Boundary and S. Uplands faults but these are much less well defined and have less associated mineralisation. Many questions must be answered, however, before the association between the geomagnetic data and possible subduction zones can be made with confidence in Scotland. Further field observations, already in progress, should enable both the location and depth of the conductive zones to be located more precisely. Magnetotelluric measurements at locations traversing the anomalous regions in N. Scotland—planned to start in 1977—should indicate whether the Z contours should be associated with single or complex conductors. Recent magnetotelluric measurements in S. Scotland should assist in the interpretation of the regions D and E. Our results suggest a more complicated geometry for the Eskdalemuir anomaly than was shown by earlier work[1,2]. There seem nevertheless sufficient

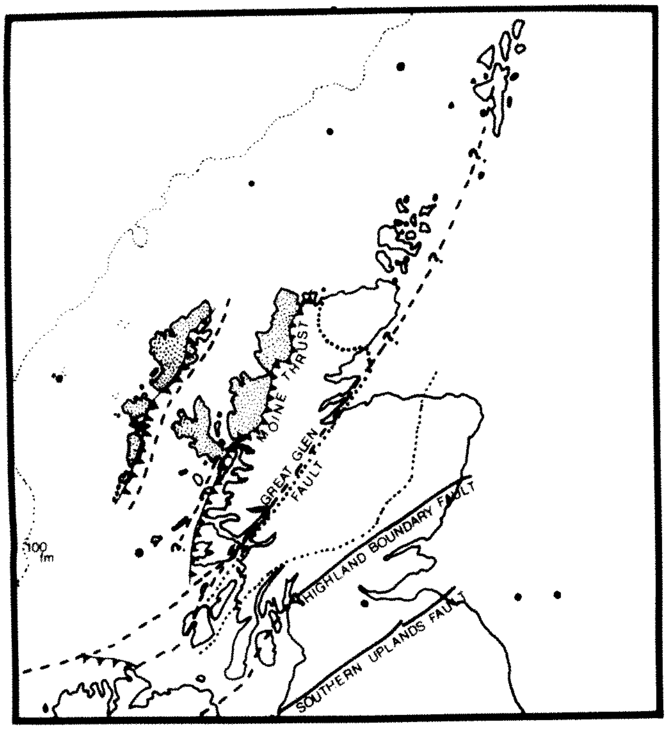


Fig. 3 Simplified tectonic map of Scotland. Shaded region is the Lewisian Foreland. Dotted curve represents ancient subduction zones proposed by Garson and Plant.

grounds to propose that there may be a close relation between the complex electrical structure of the crust in Scotland and past tectonic underthrusts.

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Tectonic and sedimentary significance of Cretaceous Tekenika Beds of Tierra del Fuego

STRATA called Tekenika Beds have been considered the oldest post-orogenic rocks within the southernmost Andean Cordillera. Their significance in terms of regional tectonic history outweighs their small outcrop area on the east side of Hardy Peninsula on Hoste Island, Chile, about 160 km north-north-west of Cape Horn (Fig. 1). The Tekenika Beds, which are 2–3 km thick, are best known on Burleigh Peninsula on the south side of Tekenika Bay (Tekenika derives from two Yahgan Indian words *Teke uneka*[1]), where they are largely sandstone and conglomerate (Figs 1 and 2). The basal contact has not been seen; however, the strata, although folded (ref. 2 and unpublished reports of Empresa National del Petroles), are less deformed than the tightly folded Lower Cretaceous Yahgan Formation just north of Tekenika Bay less than 5 km away. Therefore an unconformity is implied. Coarseness of the strata, fossil wood (some bored by worms or molluscs), thin lignitic coal beds, shallow marine molluscs and presence of some plutonic clasts led to the view that the Tekenika is a nearshore, post-orogenic sequence[2]. Suarez and Pettigrew recognise it as pre-orogenic, but still interpret it as a shallow marine deposit[3]. We report here structural evidence indicating that the Tekenika Beds are pre-orogenic and deep water deposits.

Dating of the Tekenika rocks has long seemed important in constraining the younger age limit of Andean orogenesis in Tierra del Fuego. For this reason, Halpern in 1963 collected fossils and igneous pebbles from the Burleigh Peninsula for isotopic dating. His results suggested a very equivocal palaeontological age of 'Late Cretaceous to Early Tertiary', and K–Ar dating of a gabbroic boulder suggested 116 ± 4 Myr (Early Cretaceous) as a maximum possible age[2]. An altered greenstone considered contemporaneous with the lower Yahgan was dated as 92 ± 4 Myr, which was interpreted as being close to the time of folding and metamorphism[2]. The gabbro clasts were considered derived from the Andean batholith complex just to the south-west of Tekenika Bay. Intrusives into the folded and cleaved Early Cretaceous (Neocomian) Yahgan Formation at two localities 50 km north on Hoste and Navarino Islands were also dated as 77 ± 5–81 ± 5 Myr (ref. 2).

The above evidence seemed consistent with the view that the Tekenika Beds represent a post-orogenic molasse. Because neither fossils nor isotopic dates could resolve satisfactorily exactly when the main Andean orogeny occurred between 116 and 77–81 Myr we visited, on a cruise of R. V. Hero, in May–June 1974, all the Tekenika outcrops, hoping to clarify stratigraphic relationships and discover more definitive fossils. Some fossils were found, but the only one of possible stratigraphic significance is a colonial coral fragment from conglomerate on Navidad Bay (Fig. 1). It was identified by A. G. Coates, George Washington University, as *Actinastrea* cf. *A. peruviana*, which is known from upper Aptian- to Albian-aged strata in Peru[4]. We also found *Rotalaria*, *Yoldia*?, a naticiform snail, belemnoids and *Ostrea* fragments. All of the shells and plants were contemporaneous with Tekenika sedimentation, but were swept in like pebbles. All that any of the palaeontological evidence clearly shows is that the strata are Cretaceous.

Having assumed that the Tekenika Beds post-dated the main Andean orogeny in Tierra del Fuego, we were surprised to discover structural relationships that show them to be pre-orogenic instead; that is, late Early Cretaceous to middle Late Cretaceous or post-Yahgan and pre-77–81 Myr. To summarise, Yahgan strata contain Neocomian fossils and lie below the Tekenika beds; the latter contain 116-Myr-old pebbles and a possible Albian–Aptian fossil. Folds and cleavage in the Yahgan are cut by 77–81-Myr plutons, and concordant cleavage was found in the Tekenika. Therefore, the Tekenika Beds are post-Neocomian and pre-77–81 Myr, possibly Albian or Aptian. The Tekenika Beds lie within a synclinorium whose axis trends west-north-west-parallel to more tightly compressed folds in the Yahgan Formation north of Tekenika Bay (Fig. 1). North of Burleigh Peninsula we found alternating sandstones and olive-drab-weathering mudstones concordant with darker, more indurated graywackes and mudstones of the Yahgan Formation (Fig. 2). The basal contact of the sequence could not be located precisely because of intrusive dykes and poor exposures. Similar strata occur south-east of Burleigh Peninsula along Navidad Bay, but relationships of these lower Tekenika Beds to fossiliferous Yahgan strata (or Hardy Formation of

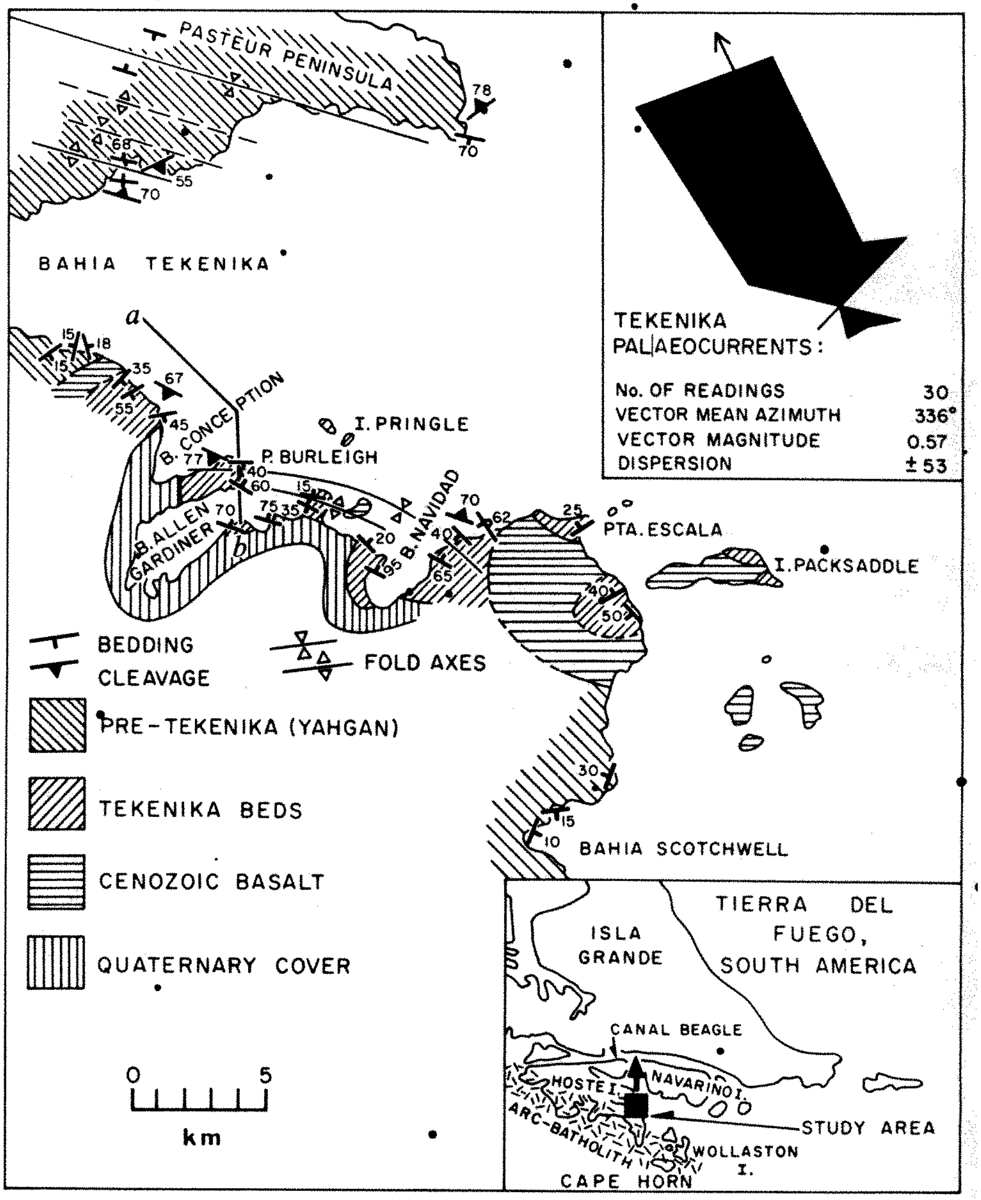


Fig. 1 Schematic geological map of Tekenika Bay area, southern Chile; pre-Tekenika at Bahia Scotchwell includes Hardy Formation of Suarez and Pettigrew[3,8]. Heavy arrow in inset is average Yahgan palaeocurrent direction.

Suarez and Pettigrew[3]) farther south at Scotchwell Bay are obscured by a late Cainozoic basal tcomplex on Punta Escala and Isla Packsaddle (Fig. 1). Our observation of a subtle but definite cleavage in the Tekenika mudstones is particularly significant. Generally the cleavage consists of sporadically developed fractures, but locally it is slaty and parallels the regional slaty cleavage characteristic of the Yahgan rocks to the north.

Throughout this part of the southern Andes, deformation and metamorphism are remarkably heterogeneous, generally diminishing in intensity southwards from a maximum along the north side of Canal Beagle[5–7]. Just north of Tekenika Bay, the Yahgan rocks are tightly to openly folded, with a pronounced slaty cleavage. South of Tekenika Bay, however, at Bahia Scotchwell, for example, folding in the fossiliferous Lower Cretaceous rocks is gentle. The rocks here have a well developed joint system, but slaty cleavage is non-existent. Thus the slight deformation of the Tekenika rocks is consistent with the regional structure. Their less deformed and metamorphosed nature is not the result of a post-orogenic age, as long assumed, but reflects its structural location. We infer that the Tekenika Beds were originally more widespread, and have been preserved due to their location in the axis of a regional synclinorium.

Sedimentologically we also found much similarity with underlying Yahgan flysch strata. Both show the same south–north palaeocurrent pattern (Fig. 1) and both are characterised by graded bedding throughout (Fig. 3); sandstones show typical Bouma sequences in both cases. The Tekenika grades upward from Yahgan-like alternating graywackes and mudstones into thick, crudely graded sandstones with less mudstone, and in turn graded and massive volcanic cobble and boulder conglomerates with some clast imbrication (Fig. 2); sandstones are poorly sorted lithic (volcanic) graywackes with considerable matrix[2,11]. The coarse volcaniclastic deposits have sedimentary structures identical with those of conspicuous andesitic breccias and coarse sandstone zones interstratified within the generally finer Yahgan on western Navarino Island (Fig. 1). Both are generally poorly sorted and show massive as well as graded units. Parallel stratification is very common and some small- and medium-scale cross bedding is present in both cases. Shallow scour structures are present, especially in the Tekenika. Fragments in the Yahgan breccias are much less rounded than in the Tekenika conglomerates, however. Both cases lack abundant medium-scale cross bedding in well-sorted sands, pronounced channelling, fining-upward sequences, extensive organic burrowing of mudstones and *in situ* coals, all characteristic of deltaic deposits. Also lacking are low-angle inclined stratification in well-sorted sands, layers with shell or heavy mineral concentrations and soil profiles characteristic of littoral deposits.

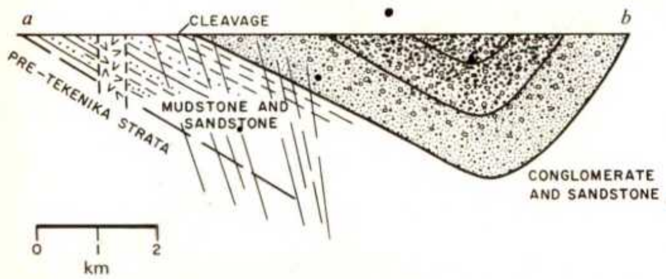
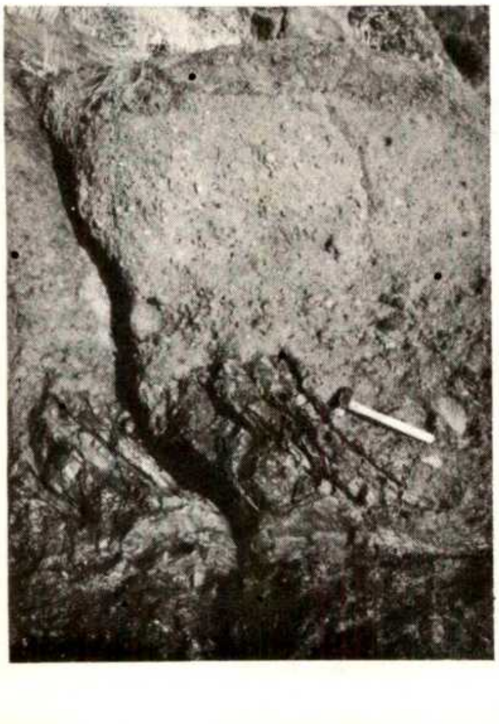


Fig. 2 Cross section of Tekenika Beds along line *a–b* shown in Fig. 1. Note cleavage and a dyke (left) penetrating both pre-Tekenika and Tekenika strata.

The Yahgan-aged (Neocomian) rocks coarsen southward toward the main Patagonian batholith; they have been named the Hardy Formation[3,8]. Although Suarez and Pettigrew included the Tekenika Beds in their Hardy formation, we consider the former a member of the Yahgan formation because it shares deeper-water characteristics, structural concordance and grade of metamorphism with the Yahgan. Sedimentology of the Hardy rocks and their relationships to the Tekenika are not yet well documented. Just south of Scotchwell Bay are pillow lavas and volcanic breccias interstratified with Yahgan-like mudstones, sandstones and volcanic conglomerates containing marine Lower Cretaceous fossils. Near the southern end of Hardy Peninsula (inland from Orange Bay) we observed apparently non-marine silicic breccias with accretion lapilli and welded tuff zones interstratified with mafic and intermediate breccias and vesicular flows. The same assemblage occurs also on the Wollaston Islands 100 km to the south-east (Fig. 1). These volcanic rocks are of uncertain age, but the simplest hypothesis is that they comprise part of the Early Cretaceous volcanic arc complex. Cross-bedding orientations within coarse green sandstones indicate south–north palaeocurrents, which agree with those of the Yahgan and Tekenika strata.

Fig. 3 Graded Tekenika conglomerate with large ripped up mudstone block at base.



There are two laterally-equivalent Cretaceous sedimentary facies in southern Tierra del Fuego, which were both derived from an arc source—the generally finer Yahgan (north) and the coarser conglomerates and breccias (south)[9]. This situation is duplicated in equivalent rocks of South Georgia Island now 2,000 km to the east, but which is interpreted as originally having been adjacent to Navarino Island[5]. Significantly, the structural situation on South Georgia and Annekov Island is also similar to that in Tierra del Fuego, in that mid-Cretaceous deformation was concentrated towards the north, while sedimentary and igneous rocks deposited in and immediately behind the volcanic arc are only gently deformed[5–7,10,11].

We interpret the Tekenika Beds to be late Early to early Late Cretaceous in age, and to represent the youngest recognisable part of a continuous coarsening-upward (and southward) volcaniclastic succession. The typical Yahgan flysch to the north comprises submarine fan deposits debouched into a relatively deep, narrow marginal or back arc basin[9–11]. We believe the Tekenika was deposited in a similar setting, but the coarse, upper Tekenika must represent an upper fan channel or suprafan lobe closer to the volcanic arc source than most of the Yahgan. The heterogeneous breccias, tuffs and flow rocks of southern Hoste and the Wollaston Islands (Hardy Formation) presumably were deposited within the arc itself; the batholiths are the roots of the arc[5,8,11]. The Tekenika conglomerates and sandstones were derived from rocks such as these, and were redeposited below wave base rather than on deltas and shorelines. This is based on the prevalence of graded bedding and the fact that all of the plant and invertebrate material has clearly been transported. Land-derived logs up to 4 m long and oriented by currents as well as shallow-marine shells occur within conglomerates and coarse sandstones; the lignitic coal occurs as lenticular seams only a few cm thick at the tops of graded units. Rounding of the Tekenika gravel indicates derivation from beach or stream gravels formed originally on volcanic islands. Rare chaotic pebbly mudstones (diamictites) indicate mass gravity flows, while the graded conglomerates of both the Yahgan and Tekenika indicate transport by less viscous turbidity currents, debris flows or other gravity-driven mechanisms. Such resedimented conglomerates have received considerable study in recent years, and are proving to be rather common in rock sequences like those of the southernmost Andes.

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Rapid removal of plutonium from the oceanic surface layer by zooplankton faecal pellets

It is possible that increasing quantities of plutonium will be introduced into the marine environment, so detailed knowledge of the oceanic behaviour of this element is highly desirable. The levels of plutonium in sea water and marine organisms have been established in a general way[1,2], and suggestions have been made as to possible transport mechanisms. Noshkin and Bowen[3] proposed a model which associates plutonium with a mixed population of particles, sinking at rates of 70–392 m yr^{-1}, and Folsom *et al.*[4] suggested that algae and, perhaps, the main phytoplanktonic biomass may have an important role in determining the chemical and physical forms of plutonium predominant in the ocean. The role of the zooplanktonic biomass has not been investigated in detail, but it is known that for several elements zooplankton metabolism may be an important biological factor in the removal of the elements from the surface layer of the ocean to depth[5–9]. The particular importance of zooplankton faecal pellets in this process has been highlighted, and the faecal pellets of a common zooplanktonic species, the euphausiid *Meganyctiphanes norvegica*, have been shown to be rich in the important naturally-occurring alpha-emitter ^{210}Po when compared with whole organism levels[10]. We have performed a similar study for plutonium, and we report here that *M. norvegica* faecal pellets are indeed relatively rich in plutonium, and suggest that zooplankton faecal pellet deposition might be an important vector in the vertical oceanic transport of the element.

The environmental levels of plutonium are extremely low, and collection of adequate sample material was a major problem; a faecal pellet collection system was devised[11] which provided us with faecal pellet samples of about 100 mg dry mass. Oven-dried samples were acid-digested, treated with hydrofluoric acid, and then subjected to previously described ion exchange techniques[12] to isolate plutonium; ^{236}Pu was used as a spike. Thin sources were prepared and counted by α-spectroscopy. Exacting chemistry was necessary to achieve consistently low 'blank' counting rates, and long counting times were commonplace. The results are given in Table 1, together with the $^{239+240}$Pu concentrations in whole euphausiids, euphausiid exoskeletons collected by dissecting 336 euphausiids, and in a bulk sample of microplankton which serve as food for *M. norvegica*. We found that the concentrations in faecal pellets, although varying widely, were two orders of magnitude larger than those in whole euphausiids and an order of magnitude higher than in the typical food organisms; plutonium levels in the faecal pellets were in fact vastly in excess of those found in typical marine biological materials[2].

What are the implications of these high concentrations in faecal pellets for the flux of plutonium through living euphausiids? A plutonium atom ingested or absorbed by a living euphausiid can be eliminated from the animal by faecal pellet excretion, by moulting, by egg production or by soluble excretion; alternatively, it can be assimilated during growth in one of the organs or tissues and remain in the animal. We calculate the flux of plutonium in a living euphausiid by using available biological data[9] for *M. norvegica*. We multiply the typical faeces production rate, 0.038 g dry faeces per g dry animal per day, by the mean plutonium concentration in faecal pellets given in Table 1. We multiply the moulting rate, 0.009 g dry moult per g dry animal per day, by the concentration in the euphausiid exoskeleton. Collection of eggs in sufficient quantity is an unrealistic task; fortunately, the egg production rate[9,10], about 0.002 g dry eggs per g dry animal per day, is low, and trace element concentrations in eggs are usually unexceptional. It is extremely unlikely that this term will contribute significantly to the flux. To estimate the loss by soluble excretion we performed experiments of a type previously described for other elements[10], using live euphausiids labelled with ^{236}Pu, and found a soluble fractional excretion rate of 0.08 ± 0.02 per day. This value multiplied by the plutonium concentration in whole euphausiids gives the flux loss by soluble excretion. Finally, the amount of assimilated plutonium is obtained by multiplying the whole animal concentration by 0.015 g dry mass increase per g dry animal per day, an average growth rate for non-larval individuals.

Table 2 Flux rates for various transfer modes of plutonium through living euphausiids*

	$^{239+240}$Pu (pCi kg^{-1} d^{-1})
Faecal pellets	5.4
'Moults'†	0.011
Soluble excretion	0.032
Assimilation	0.006

*The flux rates listed in this table and discussed in the text represent three output fluxes plus the assimilation. The sum of these four terms must of course equal the input flux rate, which represents the plutonium taken up by the animal from both water and food. No information about the input via the water route is available, and at this stage only a crude estimate of the input from food can be made. This is done by multiplying the known food ingestion rates for *M. norvegica*, namely between 0.113 and 0.320 g food per g dry animal per day (ref. 9), by the plutonium concentration in microplankton given in Table 1. The result is a 'food only' input flux rate of between 0.6 and 1.6 pCi plutonium per kg per day, which is of the same order of magnitude but less than the estimated total output flux rate of over 5 pCi per kg per day. Fairly wide variation of element concentrations from one microplankton sample to another is, however, to be expected[19]; a more specific identification of the *M. norvegica* food as well as further determinations of the plutonium content of faecal pellets will be needed before the flux balance can be checked accurately and the relative importance of the food and water intake routes assessed.

†The flux via 'moults' quoted above is of course only an approximation, since the concentration in true moults may not necessarily be the same as in the exoskeletons which formed our sample. We have not yet been able to collect euphausiid moults in sufficient quantity to detect plutonium, but we hope to do so in the future; it is, however, very unlikely that the argument which we give in the text will be altered substantially.

The results of these flux calculations are given in Table 2. The complete dominance of the faecal pellet term is immediately obvious, and indicates that 99% of the plutonium taken up by *M. norvegica* is excreted in the faecal pellets. Euphausiid pellets sink fairly rapidly[13], at rates ranging from 126 to 862 m d^{-1}, and they decompose relatively slowly[8]; it seems clear that they have the potential to reach the sea bottom in most areas. If the *M. norvegica*

Table 1 Concentrations of $^{239+240}$Pu in zooplanktonic material and microplankton

Sample	Wet–dry weight ratio	Individual determinations* pCi kg^{-1} dry	Mean pCi kg^{-1} dry
Whole euphausiids	4.7	0.32 ± 0.08; 0.47 ± 0.08	0.4
Euphausiid exoskeleton	4.3	1.2 ± 0.4	1.2
Euphausiid faecal pellets†	4.4	310 ± 50; 76 ± 8; 39 ± 14	142
Microplankton‡	10.7	4.9 ± 0.8	4.9

*Each individual determination is in fact the mean of two determinations made on the two halves of a split sample.

†High concentrations of certain trace elements[9,18] and radionuclides[19] in faecal material brought about by biological amplification processes have been noted previously. The fact that the plutonium concentration in the faecal pellets is higher than that in the food is simply a reflection of a high discrimination factor against a biologically non-essential element.

‡Principally small crustacea, phytoplankton and detritus collected with 65-μm mesh size plankton net along with the euphausiids.

data can be considered as typical of the marine zooplankton biomass as a whole, we can make a rough estimate of the removal time[10] of plutonium from the upper mixed layer of the ocean by zooplankton faecal pellets alone. We multiply the faecal pellet flux rate from Table 2 by 10^{-4} kg m^{-3}, a reasonable, if controversial, estimate[10,14–16] of the dry zooplanktonic biomass in the upper mixed layer, and divide this product into 0.7 pCi m^{-3}, a typical value (ref. 2) of the $^{239+240}$Pu concentration in surface sea water. The result is 3.6 yr, a figure which one would like to compare with estimates of the removal time of plutonium from the upper mixed layer by all routes. Unfortunately, direct quantitative estimates of this quantity are not available; the indications[3,17] are, however, that it is short—of the order of 1 yr. Our estimate for the removal time due to zooplanktonic faecal pellets alone is of the same order, suggesting that faecal pellet removal has a significant role in the removal of plutonium from the surface layers of the sea. Investigations of possible competing removal routes (for example sinking phytoplankton detritus, large inorganic particulate settling) are needed to substantiate this suggestion.

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Growth rate and longevity in *Drosophila melanogaster* and *Tribolium castaneum*

THERE are two main groups of hypotheses concerning the mechanism of ageing and death. The stochastic hypothesis assumes that ageing is due to the progressive deterioration of the metabolic machinery caused by the random accumulation of disturbances in one or the other of the cellular macromolecules. This hypothesis has been subject to many experimental tests, but is not yet supported by conclusive evidence. The developmental hypothesis of ageing considers senescence and death as the programmed ultimate stage of differentiation and development. Few experiments have been designed to test that last hypothesis. In two species of insects, *Drosophila* and *Tribolium*—classical tools in genetical research—we tried to verify the hypothesis which relates development to ageing. In *Drosophila* we looked at the variations of longevity linked to experimentally induced variations of one of the important components of development, namely growth rate. In *Tribolium,* we studied variations in longevity associated with the natural variations of growth rate of various ecotypes. In both cases there are clear relations between pre-imaginal growth rate and adult longevity.

Ageing and death, at the level both of the cell and of the organism, are generally considered to be the consequences of a loss of molecular information. Information loss is considered as either the result of random events or as linked to development and therefore as the programmed endpoint of differentiation. Certain previsions of the modern stochastic hypothesis—the error–catastrophe theory[1]—have been confirmed by experiment, but available evidence remains inconclusive[2,3]. The developmental theory of ageing on the other hand is not easily tested by experiment. Yet as early as 1963, Muller[4] stated that spontaneous ageing is part of normal development, that is, that development is a continuing process of which senescence forms the last stage or, in other words, that ageing is a built-in consequence of differentiation. From the latter considerations it has been deduced, correctly but probably incompletely, that the only likely way of prolonging lifespan involves a stretching of the developmental programme, essentially through a prolongation of immaturity[5]. Such a prolongation of total lifespan through a serious prolongation of immaturity, but at the sacrifice of part of the adult longevity, is not entirely unknown; it was repeatedly obtained, for instance in rats receiving various incomplete diets during adolescence[6,7]. We may ask, however, how far does interference with the development and differentiation of an organism during adolescence have any paramount effect on the future and latest steps of life? Indeed at that stage of life, differentiation and development are almost complete. Interference during the earliest embryonic stages of life seems more promising. Poikilotherms thus seem more suitable than homeotherms for use in testing a programmed senescence theory of ageing, simply because the early developmental homeostatic mechanisms of poikilotherms are not strictly regulated, although there is good evidence for temperature acclimation.

The adult longevity of a single genotype of *D. melanogaster,* samples of which had been grown in a large array of pre-imaginal environments known to induce variations in both size and duration of development, was measured in a single controlled environment. The growth rate, estimated by the ratio of thoracic size to duration of development, was negatively and highly significantly correlated to longevity[8] (Fig. 1*a*). In explaining the results the authors supposed that adult longevity is determined early in life by a defined programme. They further argued that this programme and growth rate could be concomitantly modified by varying the preadult environment. These findings apparently imply that within a species the variations in growth rate, characteristic of the various races of that species, should be reflected in the variations of the average longevities of those races. To test this deduction we studied the growth rate and adult longevity of various genotypes of *T. castaneum*—wild strains from wide geographic origin[9]—in a single controlled environment.

The environmental conditions were 33 °C, 70% relative humidity and standard medium of 95% whole wheat supplemented with 5% dried yeast. Growth rate was measured as the weight of the larva at a fixed time, the thirteenth day of the larval stage. Adult longevity was estimated during a maximum period of 227 d. Figure 1*b* shows the relationship between preimaginal growth rate and adult longevity of eight wild strains of *T. castaneum.* The variations in both traits are quite large and the relationship between them is positive and highly significant ($r=0.833$; $n=8$; $P<0.01$). It should be emphasised that there is no strong correlation between longevity and development time ($r=0.569$; n.s.) or adult weight ($r=-0.188$; n.s.) (ref. 9).

Thus in *Drosophila* an artificially obtained decrease in growth rate of a single hybrid genotype results in an increase in adult longevity, whereas in *Tribolium* the natural variations in growth rate of various wild genotypes are positively correlated with longevity. Except for those differences in experimental approach *Tribolium* and *Drosophila* differ at least in three major ways directly related to growth rate and ageing. First, duration of development in *Tribolium* seems to be under polygenic control[10], whereas in *Drosophila* selection for fast or slow development is generally unsuccessful[11]. Second, the imaginal longevities of both species are quite different; in optimal conditions the average longevity of *Drosophila* is about 50 d but in *Tribolium* it is rather more than 130 d. Third, and most importantly, in contrast to *Drosophila*[12], somatic cell division apparently occurs during the imaginal stages of the life cycle of *Tribolium*[13].

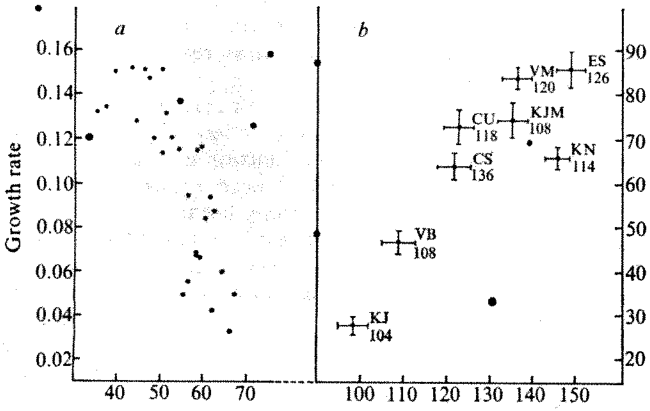

Fig. 1 *a*, Adult longevity as a function of growth rate in *Drosophila* ($r = -0.801$; $n = 28$; $P < 0.001$). Growth rate is estimated as the ratio between the cube of the thoracic size and the duration of development. Longevity is measured at 25 °C on 14 samples of two reciprocal hybrids raised in various pre-imaginal environments—six different temperatures, eight different pre-imaginal population densities—which are known to modify both the size and the duration of development of the treated individuals (redrawn from ref. 8). *b*, Adult longevity ± standard error plotted against growth rate, measured as mean 13-d larval weight, of eight wild strains of *T. castaneum*. The strains are identified according to their geographic origin as: CS (Capetown, South Africa), CU (Chicago, USA), ES (Edinburgh, UK), KJ (Kyoto, Japan), KJM (Kingston, Jamaica), KN (Kano, Nigeria), VB (Vicosa, Brazil) and VM (Veracruz, Mexico). Longevity measurement is based on observing adult death every 10 d up to a maximum of 227 d from egg. This period covered most of the adult death for all strains and the percentage of adults still alive after this period (with a maximum of 18% for ES) is highly correlated with the longevity estimates ($r = 0.840$; $n = 8$; $P < 0.01$). Numbers indicate the sample size of each strain.

In *Drosophila* longevity thus seems to depend negatively on growth rate. Growth rate is the productivity per unit of time, the resultant, due to genotype-environment interactions, of anabolism and catabolism. Growth rate may be associated with mitotic division rate. When growth rate is accelerated or decelerated it may, as a first approximation, be admitted that the same happens for mitotic division rate. Bozcuk[12] has demonstrated that the tissues of *Drosophila* show no mitoses after emergence of the imago, so the death of the organism must, to a certain extent, coincide with cellular death, or at least with the death of a certain number of cells.

It is known from studies carried out *in vivo* in higher organisms that, when a cell enters the ageing pathway, it has a life expectancy which is characteristic of the tissue[14]. When the mitotic rate of the tissue is high, as in the intestinal lining, the life expectancy of the functional cell is only a few days; when it is moderate, as in epidermis, the life expectancy may be a few weeks; and when it is low, as in liver, the life expectancy may exceed 1 yr. Furthermore, artificial prolongation of the epidermal cell life, and of the liver cell life, may be obtained in conditions of stress which provoke a decreased mitotic rate[14]. These relationships between growth rate, mitotic division rate and longevity do not necessarily demonstrate the validity of the developmental theory of ageing, nor do they exclude the error-catastrophe theory. On the one hand, in relation to the developmental theory it may be supposed (as was done to explain the influence of preimaginal temperature on adult life-span[15]) that the growth rate affects the type of molecules that are synthesised and the rates at which these molecules carry out metabolism. If the idea of morphogenes[16] is accepted, it may then be supposed that variations in the type of molecules present in a cell affect the course of differentiation and development. Variations in the molecular composition of a cell as a function of growth rate are not unknown; in *Escherichia coli* for instance the protein composition of the ribosomes varies as a function of growth rate[17]. A changing growth rate could thus result in a different cytoplasmic molecular composition or configuration which could explain an earlier or later programmed death of the cells and of the organism. On the other hand, if a slower mitotic rate is accompanied by a lower number of errors of replication (a point which as yet has not been demonstrated) this could explain a later, randomly provoked unprogrammed cellular and organismic death.

In *Tribolium*, where mitotic divisions do not cease after emergence[13], growth rate is, in contrast to *Drosophila*, positively correlated with adult longevity. So it seems that a faster growth rate, resulting probably in a shorter cellular life expectancy, must be associated in a certain number of stem cells with a higher potential number of cell divisions. The death of the individual will not coincide with the death of some particular cells but with the end of mitotic divisions in particular stem cells. Although the problem has been studied, little is known about the *in vivo* limited or unlimited lifespan of particular stem cells[18]. One may thus hypothesise that a modification in mitotic rate, through a modification of an asymmetrical type of mitotic division[19], may result in a modified cytoplasmic molecular composition or configuration, with possible implications on cellular fate.

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Albedo measurement for remote sensing of crop yields

Any remote sensing programme designed to estimate regional agricultural productivity has three major requirements. First, the crops present must be identified; second, the amount of land planted to each type must be determined, and third, the yields of the crops per unit land area must be evaluated. The large area crop inventory experiment (LACIE) uses measurements of reflected solar radiation to fulfil the first two requirements in a true remote sensing context, but to establish yield, LACIE relies on statistical and climatological models largely dependent on ground-based observations[1]. Here we report the basis for a crop yield prediction programme that is not subject to this restriction. Working with wheat, we have shown that the intensity of reflected solar radiation can be used to evaluate crop yields per unit land area. Linked to a satellite system, this could make the worldwide surveillance of agricultural production a technological reality that could greatly stabilise erratic fluctuations in world grain trade of the type which have recently disturbed the world economy.

On 3 December 1975, a 72 × 90-m field of Avondale loam that had been given an initial application of nitrogen fertiliser was planted with spring wheat (*Triticum durum* Desf. variety Produra). The field was then divided into six nearly equal north–south oriented rectangular plots. Five days after planting, each plot was irrigated with 10–12 cm of water. There was no further irrigation until 83 d after planting, when a programme was started to develop six soil water content regimes, as shown in Fig. 1. Each irrigation usually supplied about 10–12 cm of water. The few rains supplied a total of 4.7 cm.

The albedo data plotted on Fig. 1 were obtained from measurements of incoming and reflected solar radiation over each plot, as determined by one upright and six inverted Eppley pyranometers located about 50 cm above the top of the crop canopy. The data were recorded every 20 min, summed to give daily totals to determine mean albedo values, and then normalised as described before[2] to remove variability caused by the changing altitude angle of the Sun during the growing season.

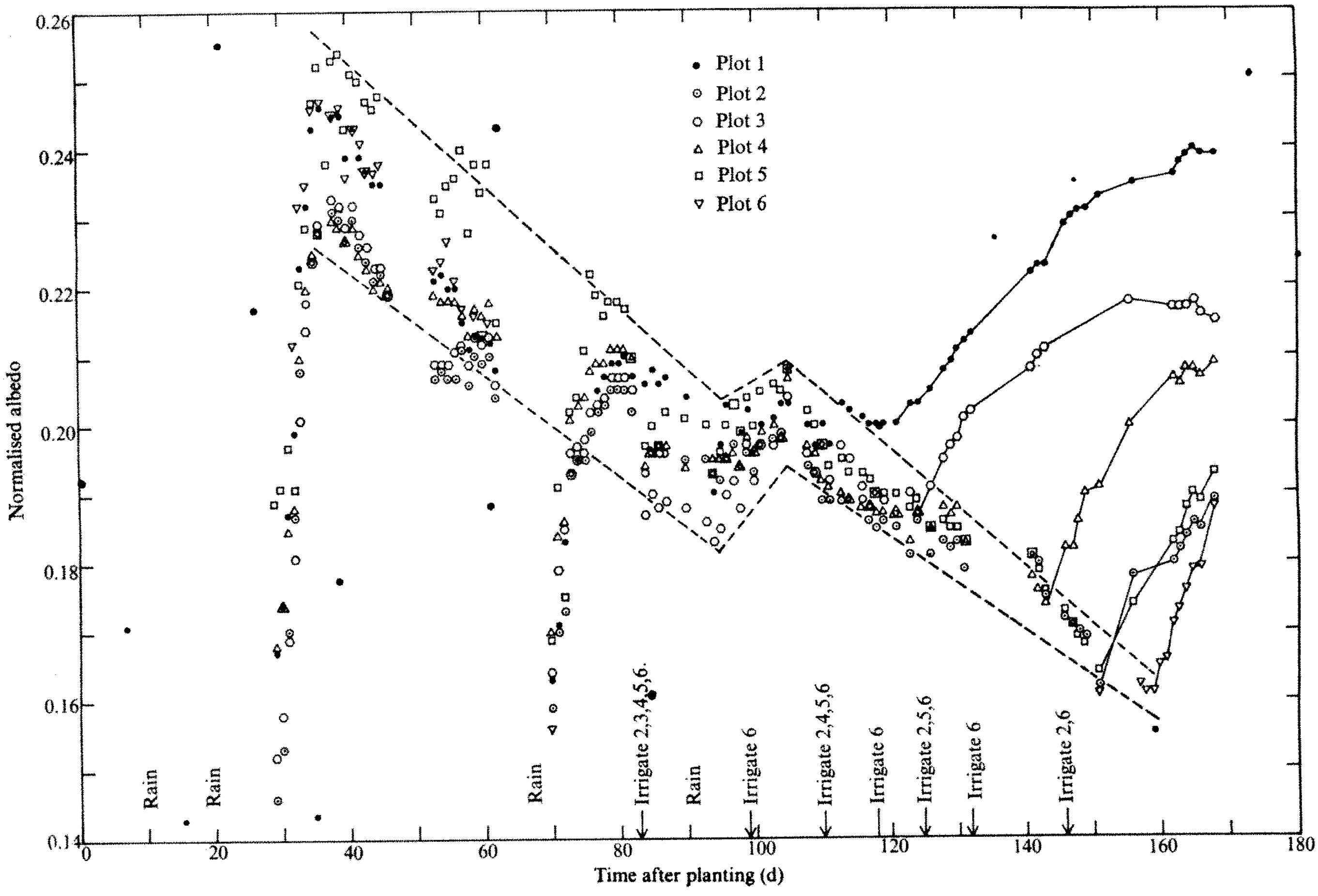
Twice weekly, green leaf area index (the ratio of green leaf area to the area of soil supporting its growth) was measured on three randomly selected plants per plot with an optically integrating area meter. When the heads emerged, three other randomly selected plants from each plot were sampled to determine mean head weight after driving off moisture by drying in an oven. To express these data per unit soil area, as in Fig. 2, at the end of the growing season we hand-harvested wheat from 14-m^2 sections of each of the six plots and counted every plant to obtain the number per unit soil area.

Figure 1 reveals a decline in albedo as the season progresses. Figure 2 shows that this is due initially to the growth and proliferation of the leaves (as they progressively shut out more and more of the high-albedo soil from view) and finally to the growth and proliferation of the plant heads (which start about day 100, when green leaf area has either reached a maximum or begun to decrease).

These relationships are shown more clearly in Fig. 3, where albedo data are first plotted as a function of green leaf area index until day 95, and then as a function of head dry weight from day 105 to near the end of head growth (when final increases in albedo began). The strong linear relation supports the idea that final grain yield is a linear function of the minimum albedo reached just before the large increases that occur as the heads begin to ripen. Comparison of these two parameters (Fig. 4) suggests that the hypothesis is verified by our data.

This analysis shows that measurements of reflected solar radiation can be used to estimate agricultural productivity in a complete remote sensing context. Our approach makes use of ground-based measurements of incoming solar radiation

Fig. 1 Clear-day normalised albedo of six differentially-irrigated plots of Produra wheat grown at Phoenix, Arizona as a function of days after planting.



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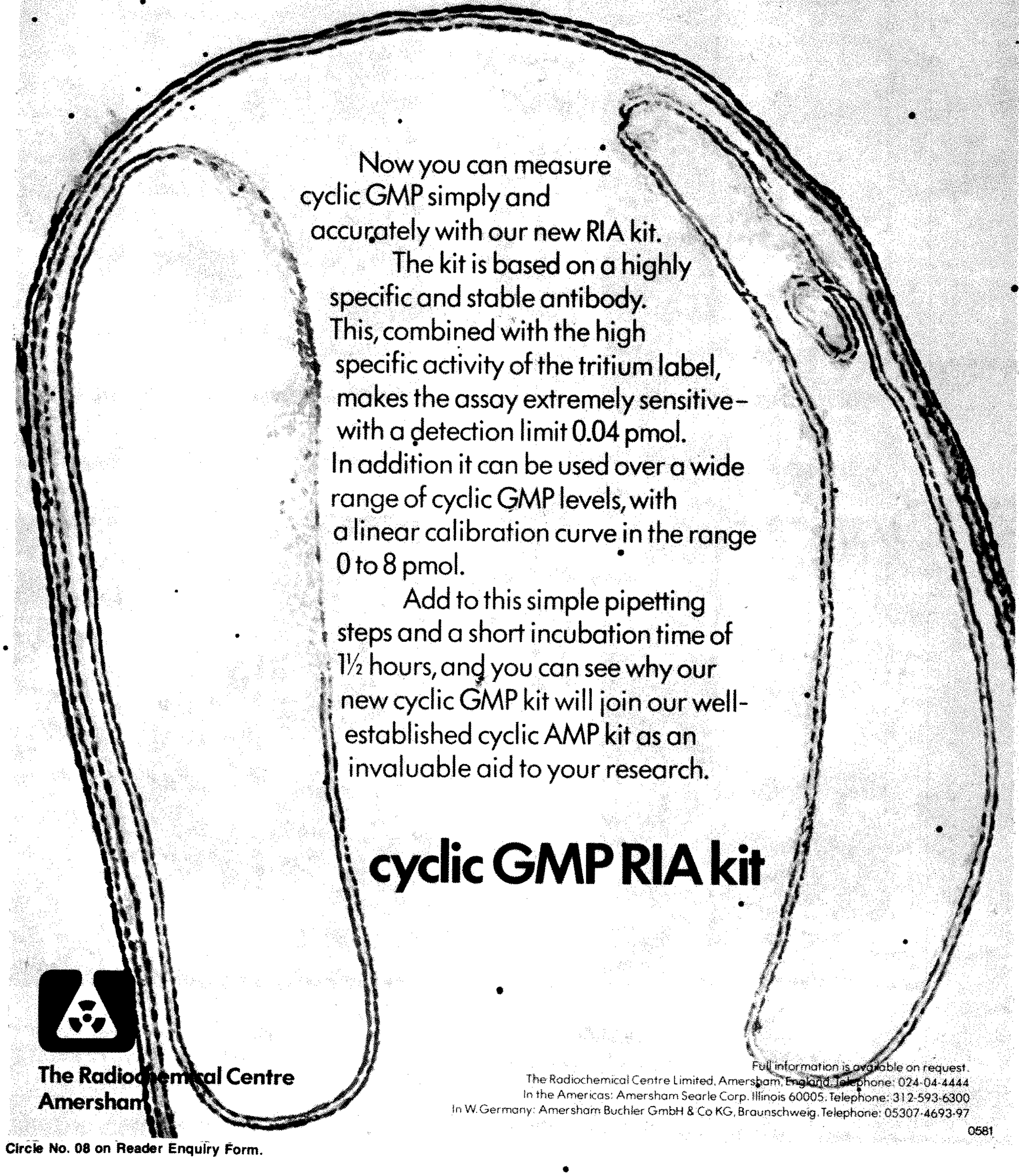

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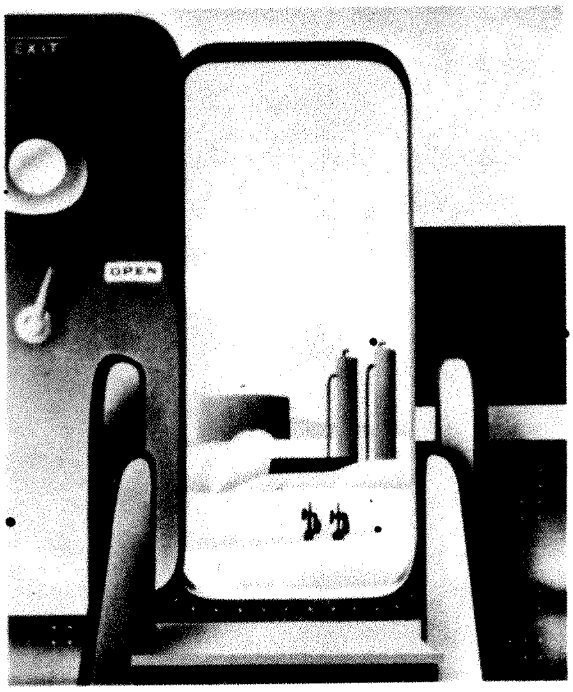

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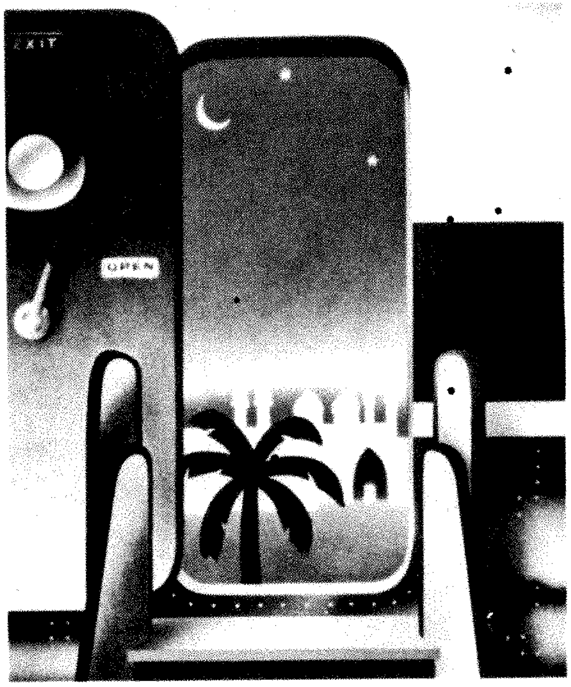

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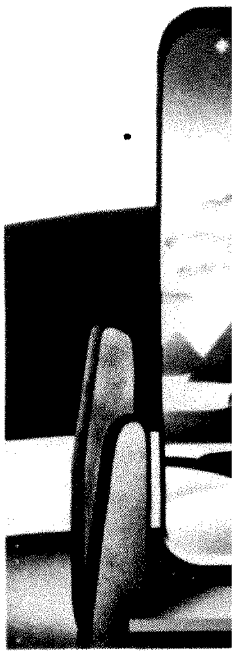

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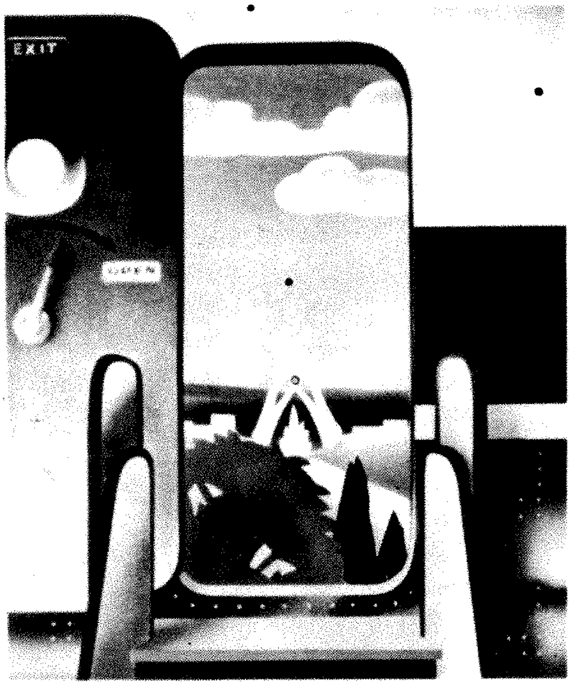

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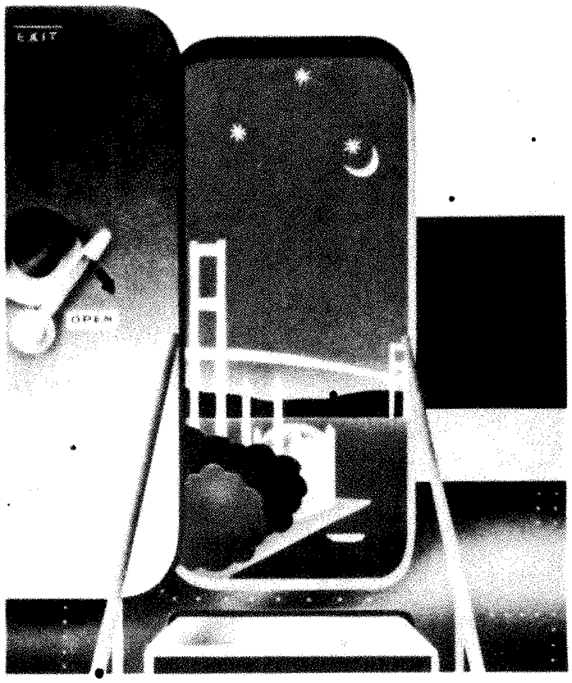

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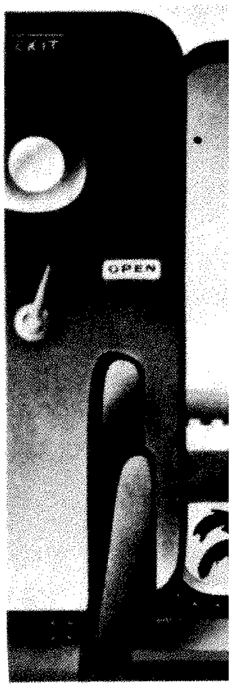

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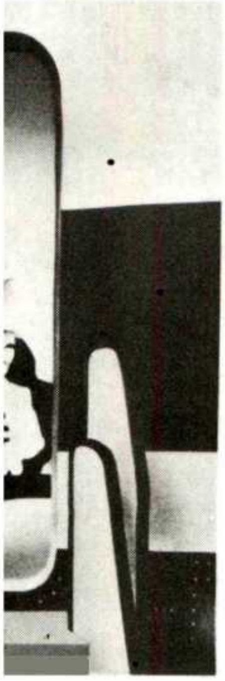

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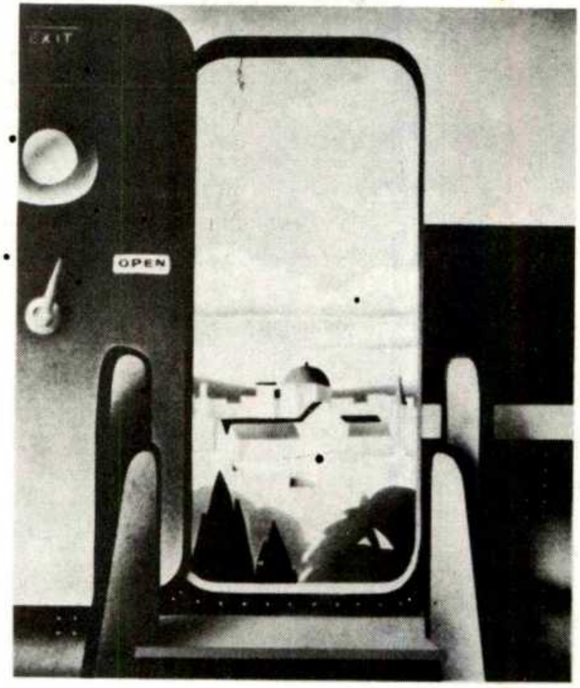

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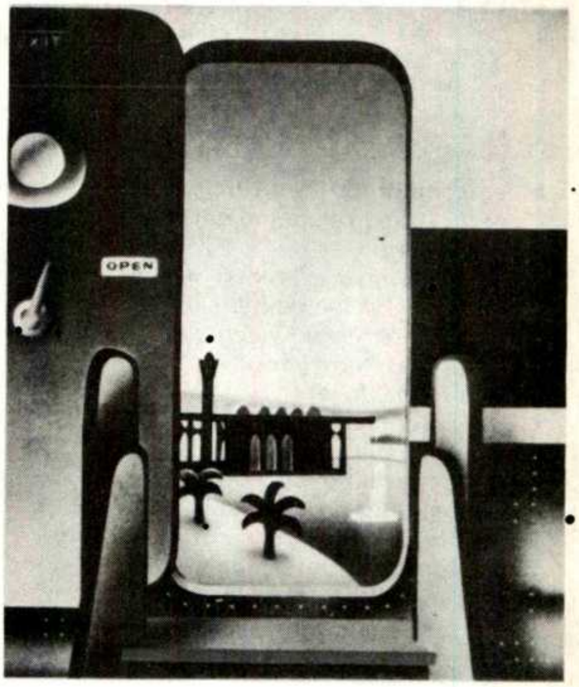

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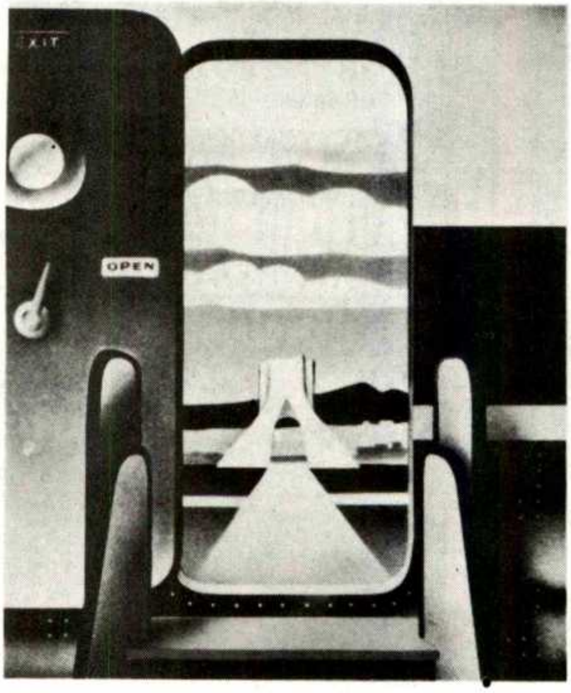

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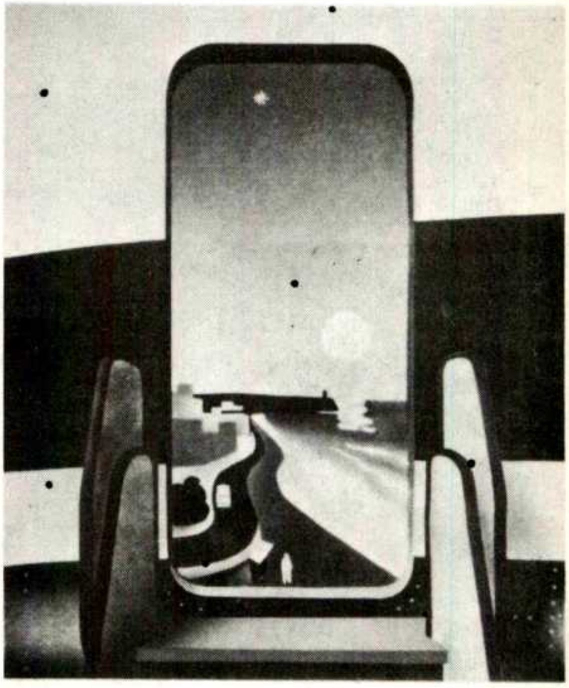

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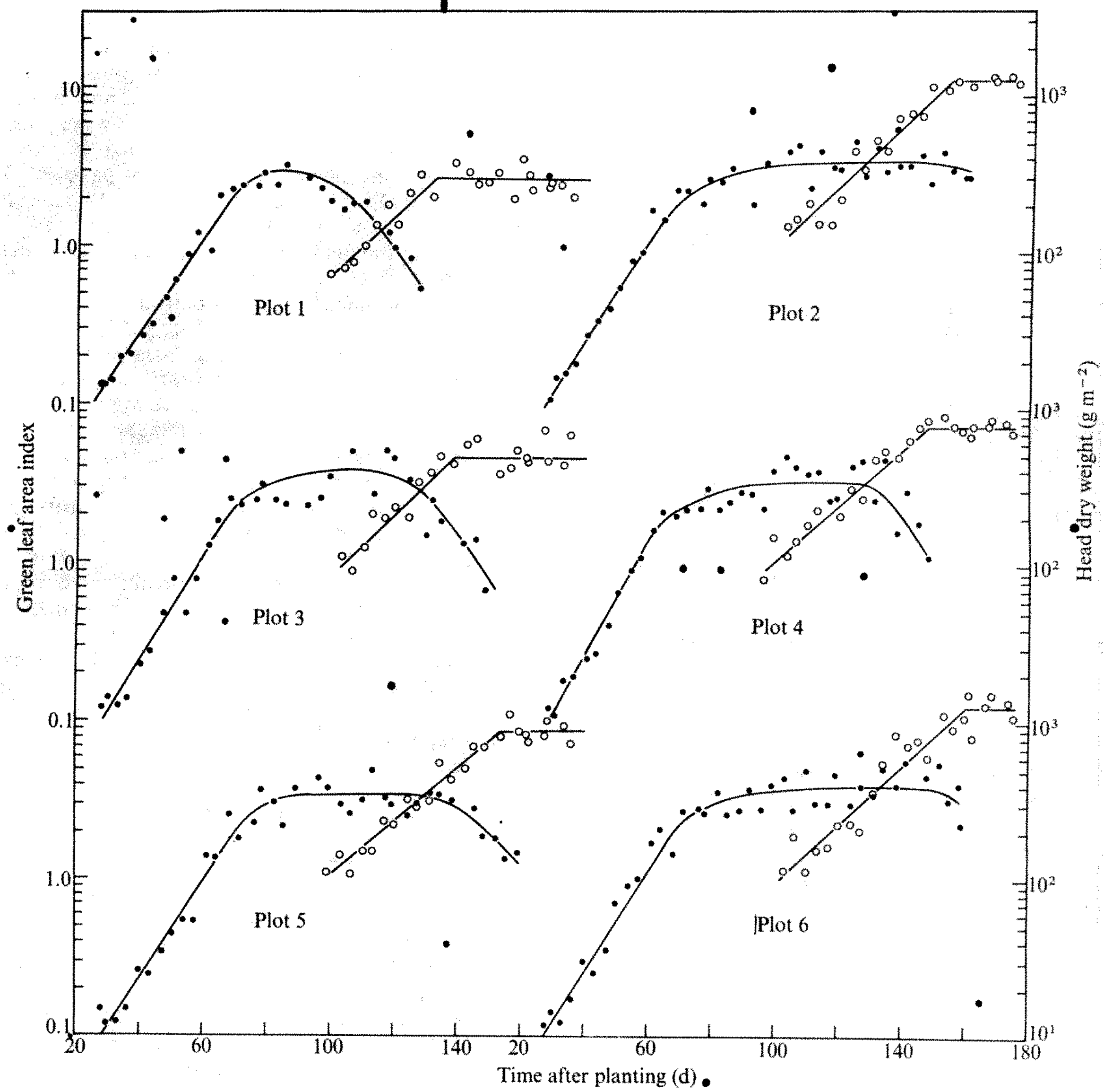

Fig. 2 Green leaf area index (●) and head dry weight (○) accumulation of six differentially-irrigated plots of Produra wheat grown at Phoenix, Arizona as a function of days after planting.

to standardise our data in an albedo format; but many other means of standardisation could be used which do not require ground-based measurements. Normalisation to some constant albedo feature such as a dry lake bed, a rock outcrop, a non-eutrophic lake, or even a man-made surface could be accomplished entirely by remote sensing. In such a programme, however, over-flights may need to be more frequent than the every-9-d coverage now provided by the LandSat satellite systems, in order accurately to establish albedo trends before and after head ripening to determine the critical minimum albedo value required to estimate final grain yield.

Our present approach is intended for use only with grain crops, but might be extended to other types of crops. For two reasons, our data suggest that the linear relationship shown in Fig. 4 may be applicable to durum wheat (Produra) grown anywhere in the world. First, the low albedo values after the early rains that reduced soil albedo were not apparent beyond day 80, when all plots had developed green leaf area indices of 2.5 or more. Thus, beyond this stage of growth, soil albedo seems to be of no consequence. Second, there were significant differences in the numbers of heads produced by each plot, yet no explicit accounting for this fact was required by us in correlating albedo with final grain yield. Thus, density of planting may also prove to be of little consequence.

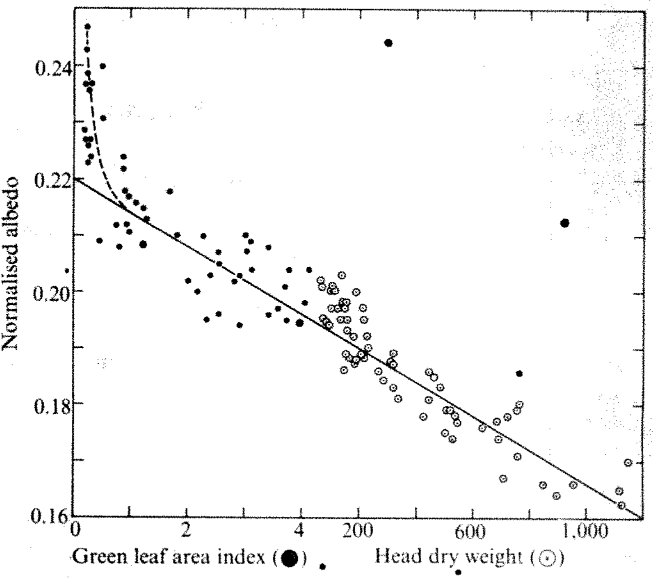

Fig. 3 Clear-day normalised albedo against green leaf area index and head dry weight of Produra wheat grown at Phoenix, Arizona.

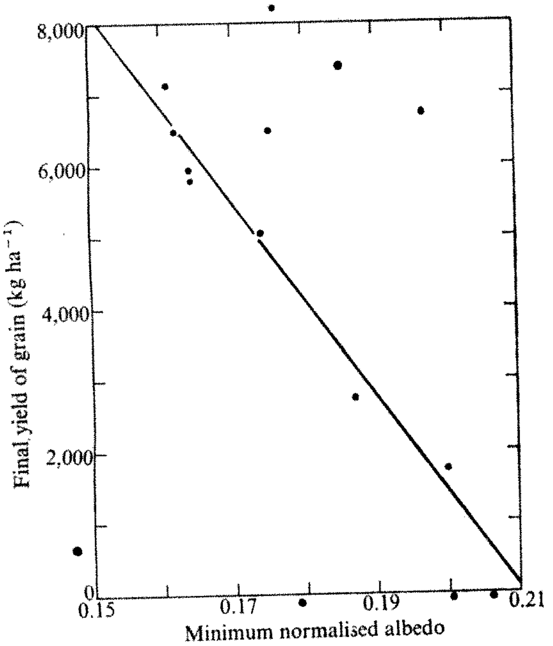

Fig. 4 Final grain yield of six differentially-irrigated plots of Produra wheat grown at Phoenix, Arizona against the minimum value of normalised albedo measured over each plot. $y = 28,370-134,700\,x$, $r_{y-x} = -0.986$.

Finally, we believe that measurement of reflected solar radiation is one viable means for the remote sensing of crop yields. Together with estimation techniques based on remote surface temperature measurement[3], it should make possible a remote sensing system for the evaluation of agricultural productivity on a global scale.

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Experimental transmission of *Leishmania chagasi*, causative agent of neotropical visceral leishmaniasis, by the sandfly *Lutzomyia longipalpis*

The phlebotomine sandfly *Lutzomyia longipalpis* Lutz & Neiva (Diptera: Psychodidae) has long been suspected to be the vector of *Leishmania chagasi* Marques da Cunha & Chagas, in the principal foci of human visceral leishmaniasis ('kala azar') in Latin America[1]. In general, the distribution of this sandfly coincides with the human disease, and the insect is a common domestic or peridomestic species which feeds avidly on man. *Lu. longipalpis* has been found naturally infected with promastigote flagellates which could have been those of *L. chagasi*[2–3], but on none of these occasions was the parasite inoculated into susceptible laboratory animals and its true identity remains unknown. Previous attempts to experimentally transmit *L. chagasi* by the bite of *Lu. longipalpis* have failed[4]. We report here the first experimental transmissions of the parasite by laboratory-bred *Lu. longipalpis*, thus providing further evidence that this sandfly is a major vector of American visceral leishmaniasis.

In transmission experiments we used an isolate (L.d./PP-75) of *L. chagasi* obtained from a child from Ituaçu, Bahia State, Brazil. The parasite was passed from the original culture medium in which it was isolated, directly into hamsters: it had been passaged in these animals on only three occasions before the present experiments were commenced. It is a particularly virulent organism in the hamster, the animals usually dying of a fulminating infection in 5–7 months after the intraperitoneal inoculation of spleen suspension from infected animals.

Our closed colony of *Lu. longipalpis* was established from flies collected in houses and farm buildings in Morada Nova, Ceara State, north-east Brazil: at the time of this work it was in its twenty-third generation[5].

The infection rate obtained by feeding *Lu. longipalpis* on the skin of anaesthetised, infected hamsters was low (about 10%) and we felt that if successful transmission was to be achieved we needed much larger numbers of heavily infected sandflies than this method could provide. Following the findings of Adler and Theodor[6], one of us (R.D.W.) tried feeding *Lu. longipalpis* on defibrinated rabbit blood through a variety of membranes. It was found that large numbers would readily engorge, and that the most suitable membrane was freshly removed chick skin. Temperature of the blood was maintained at 37 °C by use of an immersion thermostat apparatus (Thermomix II). We felt, therefore, that by using this system with a suspension of amastigotes of *L. chagasi* in rabbit blood we could probably ensure that all fed sandflies were infected.

Hamsters with 5–6-month-old infections usually showed spleens that were packed with parasites. The spleen of one such animal was triturated in a glass tissue-grinder with 4.0 ml of sterile, defibrinated blood which had been inactivated at 56 °C for 20 min (Fig. 1*a*). Approximately 300 *Lu. longipalpis* were fully engorged on this suspension, through a chick membrane, and subsequently maintained in individual tubes[7].

Dissections of sandflies confirmed a 100% infection rate: by 5 d after the infective feed they were showing massive promastigote infections throughout the midgut and up to the anterior part of the cardia, where they appeared as stubby or rounded forms attached to the intestinal wall.

Each day, all those sandflies which had oviposited were released into a cage into which was placed a clean hamster, anaesthetised with Nembutal (Abbott). The animal was placed on its back and secured to a cork board with adhesive tape: its abdomen was shaved, to offer maximum surface area to the sandflies, and all re-feedings were carried out in the dark. Twelve transmission attempts were made, using batches of 4–40 infected flies. The first was on day 7 after the infective feed, and the rest on days 8–17 and 19.

Numerous flies were dissected during the experiment in order to follow the development of *L. chagasi*, and in particular to see when flagellates first gained entry to the pharynx and proboscis.

On day 5 of the infection the single fly examined showed enormous numbers of long thin promastigotes (Fig. 1*b*) in the midgut, with somewhat shorter, dividing forms in a more anterior position in the cardia (Fig. 1*c*). No parasites were seen in the pharynx, cibarium or proboscis. Two insects dissected on day 6 showed a similar pattern of development.

On day 7, one out of three *Lu. longipalpis* showed three promastigotes swimming freely in the pharynx. These parasites (Fig. 1*d*) were notably smaller than the form seen in the midgut of the same fly, and they moved with extraordinary rapidity. No parasites were seen in the proboscis of this insect, and the two other sandflies

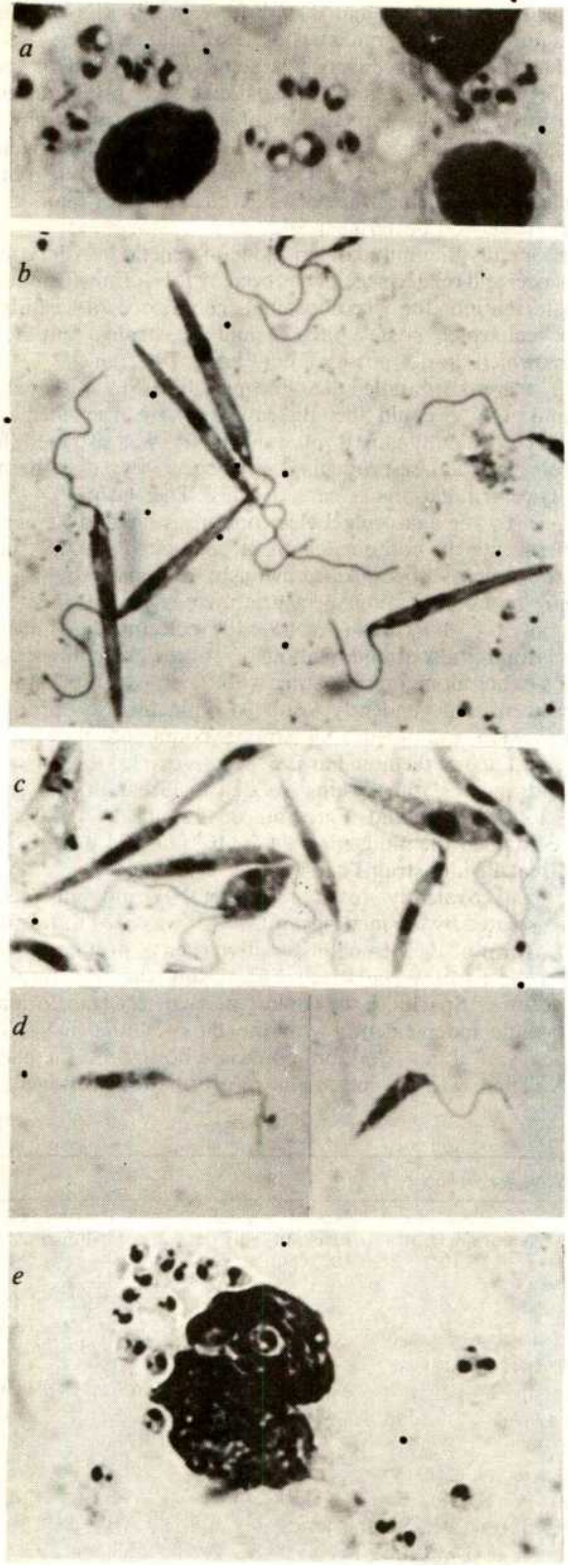

Fig. 1 Experimental transmission of *Leishmania chagasi* by the bite of the sandfly *Lutzomyia longipalpis*. *a*, Stained smear of a suspension of amastigotes of *L. chagasi* obtained by triturating the spleen of an infected hamster in inactivated rabbit blood. This material was used to infect the sandflies, through a chick-skin membrane. *b*, Long, thin promastigotes of *L. chagasi*, as seen in the midgut of *Lu. longipalpis*, 5 d after the infective feed. *c*, Shorter flagellates, some dividing, from the cardia of the same fly. *d*, Small promastigotes of *L. chagasi* from the pharynx of *Lu. longipalpis* on day 7 of infection. *e*, Impression smear of the spleen of a hamster, approximately 4 months after the animal was bitten by three infected *Lu. longipalpis*: intracellular and extracellular amastigotes of *L. chagasi*. All preparations fixed in absolute methyl alcohol and Giemsa-stained (×1,420).

dissected at the same time had flagellates no further forward than the cardia. On day 8, a single insect examined again showed a massive infection of the midgut, including the cardia, but absence of parasites in the foregut. Subsequently, pharyngeal forms were once more seen, in one fly, on day 10. Six others dissected during days 11–13 showed only midgut infections.

The first occasion on which flagellates were seen in the proboscis was on day 14, when two flies were dissected: both showed the usual, heavy midgut development of *L. chagasi* and, in addition, a number of shorter and more active forms in the pharynx. In one of the sandflies, however, a single parasite was seen moving rapidly up and down the length of the proboscis.

No foregut forms were seen in two insects examined on day 15, but light pharyngeal and proboscis infection was found in one sandfly on day 16. Finally, two flies dissected on day 17 showed parasites restricted to the midgut; no sandflies were examined beyond this date.

One hamster was found moribund, just over 4 months after three infected *Lu. longipalpis* had fed on it. Necropsy showed very large numbers of amastigotes of *L. chagasi* in impression smears of the spleen (Fig. 1*e*), and slightly fewer in the liver: culture of splenic tissues in blood–agar medium (NNN) produced growth of promastigotes. The remaining 11 hamsters were killed and examined at various dates from 5–6 months after the experiment, and four more proved to be infected: they showed variable numbers of amastigotes in the spleen, liver and bone marrow, and in all cases the spleen was the most heavily parasitised organ.

Thus at least 5 separate transmissions were achieved after feeding a total of 34 infected *Lu. longipalpis* on 12 hamsters. As up to six infected sandflies were on some occasions persuaded to feed on a single hamster, it is very likely that the number of transmissions was in fact more than five.

All transmissions were made during the first blood meal after the insects were infected, on days 7, 11, 13, 14 and 15. The transmission as early as 7 d after the infective meal is particularly interesting, for although scanty pharyngeal parasites were seen in one fly at this time no proboscis infections were recorded until the fourteenth day. Possibly the previous presence of flagellates in the proboscis is not prerequisite to transfer of the parasite, there simply being a surge of parasites forward from the pharynx, into the mouthparts, during the feeding process.

The incubation of the infection to a fatal stage in the hamster following transmission by the bite of the sandfly did not differ significantly from that obtained after the direct passage of amastigotes by syringe. This seems surprising, considering the very large number of amastigotes usually injected—with a triturate of hamster spleen, for example. It suggests that many more promastigotes may be inoculated by the sandfly vector than we have previously suspected.

We consider that the luxuriant growth of *L. chagasi* in *Lu. longipalpis*, the consistent migration of the parasite to the anterior station of the intestine and the biting mouthparts, the relative ease with which transmission was achieved by the bite of the insect, together with previous epidemiological evidence, confirm that *Lu. longipalpis* is a major vector of *L. chagasi* in endemic areas of human visceral leishmaniasis in Latin America. A detailed account of the morphological development of *L. chagasi* in *Lu. longipalpis* will be published elsewhere.

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Figueiredo was responsible for photography. Dr Robert Sogayar provided the *L. chagasi* isolate.

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Conjugal transfer of R plasmids in *Neisseria gonorrhoeae*

THE appearance of penicillin-resistant, β-lactamase-producing strains of *Neisseria gonorrhoeae* has been the cause of considerable concern[1]. We have recently shown that β-lactamase production by these gonococci is plasmid mediated[2]. Two distinct R plasmids have been characterised; one, with mass 4.4×10^6 was common in gonococci isolated from military personnel in the Far East and their sexual contacts. The other plasmid with mass 3.2×10^6 was found in a strain isolated from a woman in London[3] and we have also found this plasmid species in clinical isolates from Liverpool[4]. DNA–DNA duplex studies have revealed that both gonococcal R plasmids posses a significant portion (about 40%) of the transposable sequence, TnA, which includes the β-lactamase gene commonly found on R plasmids of the Enterobacteriaceae, *Pseudomonas aeruginosa* and *Haemophilus influenzae*[2].

While studying the β-lactamase producing gonococci from the Far East we noted that they differed in nutritional requirements and in chromosomal antibiotic resistance genes, suggesting that there had been considerable dissemination of the R plasmid rather than simply the spread of a single ancestral clone. Investigations into the possible modes of dissemination led us to discover that a 24.5×10^6 molecular mass indigenous plasmid possessing sex factor activity seems to play a significant part in the transfer of R plasmids from gonocci to other strains and bacterial species.

Historically, the only known mode of genetic transfer among gonococci and related species has been by DNA transformation[5]. Transformation for chromosomal genes occurs readily to gonococci which posses pili (T1 and T2 strains) but not to strains which do not possess pili (T3 and T4 strains)[6].

We, therefore, studied first the possibility that gonococcal plasmid DNA could be disseminated by transformation. Purified covalently-closed plasmid DNA was isolated from several β-lactamase-producing gonococci by dye buoyant density centrifugation of cleared lysates[7]. The plasmid DNA was analysed by the agarose gel electrophoresis method[8] as well as by measuring the contour length of open circular DNA in the electron microscope[2]. As previously described by us and others[10] gonococci almost invariably possess an indigenous plasmid 2.6×10^6 in mass. A 24.5×10^6 molecular mass indigenous plasmid is also found in about 5% of the penicillin-sensitive strains either alone or coexisting with the 2.6×10^6 plasmid[9].

Thirteen independently isolated β-lactamase producing strains were examined. All strains contained the 2.6×10^6 plasmid. Each of the nine Far East strains carried the 4.4×10^6 R plasmid and the four strains isolated in Great Britain carried the 3.2×10^6 R plasmid. Three out of the nine Far East strains also contained the indigenous 24.5×10^6 plasmid while none of the Great Britain strains carried it.

Purified covalently-closed plasmid DNA and bulk cellular DNA isolated by the methods of Catlin[11] was used to transform the T1 form of the penicillin-sensitive (Pen^s), proline requiring (Pro$^-$) *N. gonorrhoeae* strain F62. Using the transformation procedure of Sparling[5], we could readily detect transformation for proline independence with the bulk cellular DNA (at a frequency of about 1%)[5], but could not do so with the plasmid DNA. There was no detectable transformation to β-lactamase

Table 1 R plasmid transfer by *N. gonorrhoeae*

Donor strain†	Plasmid complement ($\times 10^6$)	Recipient strains	Plasmid complement ($\times 10^6$)	No. of penicillin-resistant cells per 10^8 recipient cells	Property of penicillin-resistant cells
N. gonorrhoeae CDC67 (T4, Pen^r) Far East	24.5 4.4 2.6	*N. gonorrhoeae* F62 (T4, *pro*$^-$ *rif*^{r} *nal*^{r} Pen^s)	2.6	10,000*	Pen^r Rif^r Nal^r Pro$^-$; 2.6×10^6 and 4.4×10^6 plasmid
N. gonorrhoeae CDC67 (T4, Pen^r) Far East	24.5 4.4 2.6	*E. coli* HB101 (Amp^s)	—	10	Pen^r *E. coli*, 4.4×10^6 plasmid
N. gonorrhoeae CDC36N (T4, Pen^r *nal*^{r}) Far East	4.4 2.6	*N. gonorrhoeae* F62 (T4, *pro*$^-$ *rif*^{r} *nal*^{r} Pen^s)	2.6	$< 10^{-8}$	—
N. gonorrhoeae IPL (T4, Pen^r *arg*$^-$ *nal*^{r}) Great Britain	3.2 2.6	*N. gonorrhoeae* F62 (T4, *pro*$^-$ *rif*^{r} *nal*^{r} Pen^s)	2.6	$< 10^{-8}$	—

Equal volumes of donor and recipient cells (1×10^7 colony forming units of each) were mixed in 10 ml of modified GC broth[9] supplemented with 2×10^{-3} M $CaCl_2$ and 2×10^{-3} M $MgCl_2$. After overnight incubation the cells were collected by passing 3 ml of the culture through a membrane filter (Biorad 0.2 µm). The filter was then placed on plates of double-strength Kellogg's medium[9] and incubated for 6 h. The filters were removed and placed in 2 ml of GC broth and vigorously agitated to remove the cells. Samples of the mating mixture were plated on Kellogg's medium supplemented with 1 µg ml^{-1} penicillin G and 1 µg ml^{-1} nalidixic acid or 1 µg ml^{-1} penicillin G and 1 µg ml^{-1} rifampicin. Colonies appearing on the selective media were restreaked five times on the same selective media, and tested for β-lactamase activity using a chromogenic cephalosporin substrate[3]. The plasmid component of representative colonies showing β-lactamase activity was determined by Agarose gel electrophoresis.

Similar mating experiments were performed with *E. coli* HB101 as recipient and β-lactamase-producing recipients were selected by plating on MacConkey's agar containing 30 µg ml^{-1} ampicillin. In some experiments, 50 µg ml^{-1} of DNase (pancreatic DNase, Worthington) was added to the broth medium at the time of cell mixing, readded just before filtration, and added once again when the cells were removed from the filter.

*The data presented is from one set of experiments. In three later matings a range of 10^3 to 10^4 penicillin-resistant cells per 10^8 recipients were found in the CDC67–F62 matings.

†T4 colony type, see text: *pro*$^-$, proline required; *arg*$^-$, arginine required; Pen^r, penicillin resistant; Pen^s, penicillin sensitive; *rif*^{r}, rifampicin resistant; *nal*^{r}, nalidixic acid resistant; Amp^s, ampicillin sensitive.

production with either the cellular or plasmid DNA preparation. A number of other gonococcal recipients were then used, including a strain devoid of any plasmid species. None was transformed for β-lactamase production. In contrast, *E. coli* K-12 recipients were transformed to ampicillin resistance at a frequency of about 10^3 cells per microgram of purified plasmid DNA, and were found to possess only a single R plasmid. We concluded, therefore, that the gonococci were refractory to transformation by closed circular plasmid DNA and the mechanism for the dissemination of the gonococcal R plasmids was not likely to be transformation.

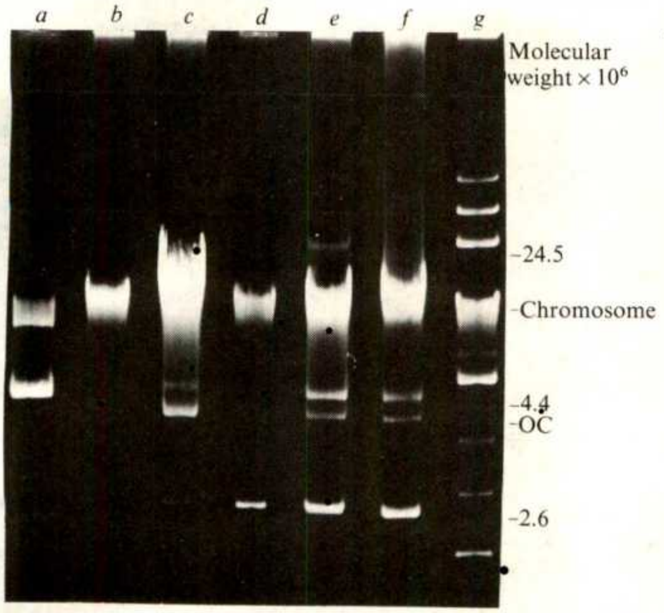

Fig. 1 Agarose gel electrophoresis of ethanol-precipitated DNA from cleared lysates of donor, recipient and penicillin-resistant recipient strains of *N. gonorrhoeae* and *E. coli*. *a*, Strain of *E. coli* HB101 penicillin-resistant recipient containing the 4.4×10^6 dalton plasmid derived from parental strain in slot *b*. Occasionally in the *E. coli* penicillin-resistant recipient lysates a plasmid band appears above the chromosome on the Agarose gel (not seen in this picture). The unusual band has been identified as an oligomer of the 4.4×10^6 plasmid by restriction enzyme analysis. *b*, Ampicillin-sensitive recipient strain of *E. coli* HB101 containing no plasmids. *c*, Ampicillin-resistant recipient containing the 4.4×10^6 dalton plasmid and the 2.6×10^6 cryptic plasmid, derived from parental strain in slot *d*. *d*, Penicillin-sensitive recipient strain of *N. gonorrhoeae* F62 containing the 2.6×10^6 cryptic plasmid. *e*, Penicillin-resistant donor strain *N. gonorrhoeae* CDC 67 containing the 24.5×10^6, 2.6×10^6 indigenous plasmids and the 4.4×10^6 R plasmid. *f*, Penicillin-resistant donor strain of *N. gonorrhoeae* CDC36N containing the 2.6×10^6 indigenous plasmid and the 4.4×10^6 R plasmid. *g*, Standard plasmid DNAs; ranging in size from 62×10^6 (uppermost band) to 1.9×10^6 (lowest band). OC refers to the open circular form of the 2.6×10^6 cryptic plasmid.

The relatively high incidence of the 24.5×10^6 indigenous plasmid among the β-lactamase producing strains suggested the possibility that this plasmid might possess sex factor activity and that R plasmid transmission between gonococci might be mediated by conjugation. Donor and recipient strains exhibiting the non-piliated T4 colonial morphology were isolated. The donor strains were all β-lactamase producers and either required arginine (Arg$^-$) or required no added amino acids to grow on Catlin's defined medium[11]. The donor strains were sensitive to 1 μg ml^{-1} of nalidixic acid (Nal) and/or 1 μg ml^{-1} of rifampicin (Rif). The recipient strain used was the T4 form of the strain F62 (Pro$^-$ Rif^r Nal^r). Matings were performed by a variation of the filter method used by Van Klingeren *et al.* to examine the conjugative properties of *H. influenzae* R plasmids[12]. Similar mating experiments were also performed using *E. coli.* strain HB101 (ref. 7) as a recipient.

The results of several representative mating experiments are summarised in Table 1. Plasmid complements of donor and recipient cells as well as resistant cells recovered from mating mixtures are illustrated in Fig. 1. β-lactamase-producing *N. gonorrhoeae* and *E. coli* recipient cells were recovered in the matings. However, successful transfer of the R plasmid was observed only when the donor gonococcal strain possessed the 24.5×10^6 plasmid as well as the R plasmid. No β-lactamase-producing recipients could therefore be isolated from matings in which isolates from Great Britain were used as donors nor with donor strains of Far East origin which lacked the 24.5×10^6 plasmid. As shown in Fig. 1, the acquisition of β-lactamase biosynthesis by recipients was associated with the appearance of R plasmid DNA. The selection of β-lactamase-producing gonococci or *E. coli* recipients was not influenced by the presence of DNse (data not shown).

The data suggests that the indigenous 24.5×10^6 plasmid plays a significant part in the transfer of R plasmids from gonococci to other strains and species. We hypothesise that this large cryptic plasmid possesses sex factor activity and is able to mobilise or otherwise promote the transfer of the smaller coexisting R plasmid by conjugation. It seems improbable that transformation is involved in the transfer of R plasmids in mixed culture because of the failure to demonstrate the phenomenon with purified plasmid DNA, because the T4 donor and recipient cells are not normally competent for DNA transformation[5], and because plasmid transfer in mixed cultures was uninfluenced by DNse. Phage-mediated transduction could be an alternative explanation for our results, although to our knowledge no gonococcal phage has been described and any transducing phage would have to have the unusual ability to infect both *E. coli* and *Neisseria*. Moreover, cell-free filtrates of the donor strains could not transfer the R plasmid to *N. gonorrhoeae* or *E. coli* recipient cells (data not shown).

It thus seems likely that conjugation is the mechanism of R plasmid transfer in the gonococci and is promoted by the 24.5×10^6 plasmid. In overnight matings the 24.5×10^6 plasmid and 2.6×10^6 plasmid are often also detected in *Neisseria* but not *E. coli* which received the R plasmid. Recent data has shown that the 24.5×10^6 plasmid is capable also of promoting the transfer of chromosomal genes. The finding that R plasmids of *N. gonorrhoeae* seem to be readily transmitted among various strains of gonococci has serious epidemiological implications. Not only must we be concerned about the possibility of widespread dissemination of resistance but also the extension of resistance to related microorganisms such as *N. meningitidis* which are currently penicillin sensitive. We feel this change is required in light of new data which has shown that in overnight mating significant numbers of gonococcal recipients received the 24.5 MDal plasmid and thus are capable of transmitting the R plasmid to new strains. This has very serious epidemiological consequences.

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Thymus metabolises progesterone—possible enzymatic marker for T lymphocytes

FEMALES of the athymic mutant strain of nude mice show severe deficiencies in reproductive function. The ovaries and the uterus are abnormally small[1], puberty is delayed[2], oestrous cycles are abnormal and mature females are usually sterile[1,3]. These abnormalities can be prevented by grafting thymic tissue at birth[2,3]. Similar abnormalities result from neonatal thymectomy of normal female mice, and can be corrected by thymus re-grafting up to 7 d age[2,4]. These observations suggest an involvement of the thymus in gonadal development and function. Conversely, certain sex hormones are known to cause atrophy of the thymus[5]. Hitherto, however, there has been no evidence implicating the thymus of the infantile mouse in sex hormone metabolism. We report here the presence of 20α-hydroxysteroid dehydrogenase (20α-SDH) activity in the mouse thymus, an enzyme engaged in progesterone metabolism.

Thymus glands of 10-d-old C57BL female mice were homogenised in 0.01 M sodium phosphate, *p*H 7.4, 0.25 M sucrose and 0.001 M $MgCl_2$ and incubated at 37 °C with 1,2,6,7-^3H-progesterone 1×10^5 d.p.m. (specific activity 100 Ci mmol^{-1}, New England Nuclear) and varying amounts of unlabelled progesterone in the presence of 1 mM NADPH and 10 mM glucose-6-phosphate. After 5–60 min of incubation the resulting steroids were extracted with diethyl ether and separated by thin-layer chromatography in the system ether–chloroform (3:10), where the substrate is separated from reduced metabolites of progesterone. Radioactive zones were located on the plates by scanning with a gas-flow counter (Packard model 7201) and the radioactive compounds were scraped off the plates, eluted with methanol and counted in a Packard TriCarb Scintillation counter.

Radiolabelled progesterone was converted to a radioactive product with chromatographic mobility ($R_f = 0.33$) identical to that of 20α-hydroxypregn-4-en-3-one (20α-OHP) and distinct from the residual substrate ($R_f = 0.66$). The compound did not dissociate from carrier 20α-OHP when cochromatographed in two additional systems, benzene–acetone (4:1; $R_f = 0.4$) and cyclohexane–ethyl acetate (1:1; $R_f = 0.46$). A mixture of the TLC-purified radioactive product (92,000 d.p.m.) and authentic 20α-OHP (31.5 mg; Ikapharm) was recrystallised thrice from aqueous methanol, yielding crystals of constant specific activity (2610 ± 130 d.p.m. mg; mean ± s.e.m.), and the specific activity of the second and third crops of crystals did not differ significantly from the corresponding mother liquors. These results indicate that the progesterone metabolite formed by the thymus tissue was 20α-OHP.

The thymic enzyme catalyses the reaction in both directions: incubation of thymus homogenate with 20α-OHP in the presence of NADP resulted in the production of progesterone (data not shown). When 20α-OHP was incubated with thymus homogenate for 2 h in the presence of NADPH, no additional metabolites were detected.

Activity of thymic 20α-SDH was linear up to 30-min incubation at 37 °C (Fig. 1*a*), and was directly proportional to protein concentration (measured by the method of Lowry *et al.*[6]), up to 600 μg ml^{-1} (Fig. 1*b*). The apparent K_m of the thymic 20α-SDH was 21.6 ± 3.3 μM (Fig. 1*c*), which is close to the value reported for the ovarian 20α-SDH (ref. 7). The V_{max} of the thymic enzyme for the reductive step was 10.0 ± 0.4 nmol 20α-OHP per mg protein per 20 min.

A comparison of 20α-SDH activity in various organs of immature mice (Table 1) shows that enzyme activity of the thymocytes is at least 16-fold higher than in lung or kidney tissue. The thymocytes seem to be the source of the thymic enzyme, as their specific activity is approximately twice that of the whole gland. Considerable enzyme activity is present in the spleen of normal C57BL mice, but is almost absent in the spleen of athymic (nude) mice (Table 1). These results suggest that 20α-SDH is associated with thymus-processed T lymphocytes, and not with B lymphocytes. To clarify this point, the ability of thymocytes and spleen cells from phenotypically normal heterozygous (nu/+ C57BL) to reduce progesterone was compared to that of spleen cells from athymic (nu/nu) mice of the same age (6-week-old). The red blood cells were selectively lysed by treatment with 0.83 NH_4Cl and the remaining white cells were washed with phosphate-buffered saline, collected by centrifugation at 500*g* (10 min) and resuspended in RPMI1640 containing 1.0 μM of labelled progesterone (400,000 d.p.m.).

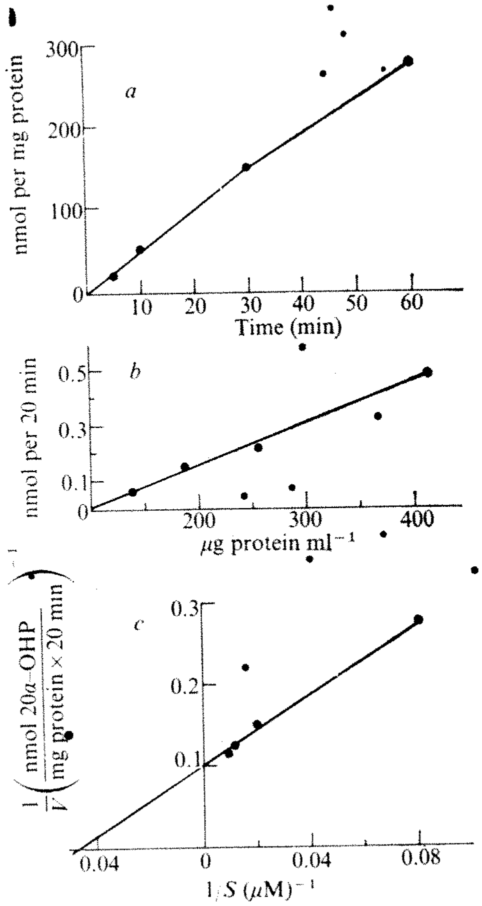

Fig. 1 Kinetic properties of thymic 20α-hydroxysteroid dehydrogenase. The enzyme in homogenates of thymus glands from 10-d-old mice was assayed as described in the text, except that in *a* and *b* cold progesterone, 25μM, was used as substrate. *a*, Time course of enzyme activity (470 μg protein per ml). Each point represents the mean of duplicate determinations. *b*, Effect of enzyme concentration on the rate of conversion of progesterone to 20α-dihydroprogesterone. *c*, Double reciprocal plot of results according to the Lineweaver–Burk method. The apparent K_m was estimated according to Wilkinson[14]. The K_m was 21.6 ± 3.3 and V_{max} 10.0 ± 0.4 nmol 20α-OHP per mg protein per 20 min.

Table 1 Distribution of 20α-hydroxysteroid dehydrogenase

Organ	Specific activity (nmol 20α-OHP produced per mg protein per h)
Thymus	4.39
Thymocytes	9.87
Spleen	0.25
Spleen of athymic mouse*	0.03
Lung	0.61
Kidney	0.29

Thymus and other organs from 10-d-old female C57BL (nu/+) mice were assayed for 20α-SDH activity as described in Fig. 1 legend. The homogenates were incubated for 60 min.

* Athymic nude C57BL (nu/nu) mouse and the phenotypically normal heterozygous C57BL (nu/+) were obtained from G. L. Bomholtgaard, Denmark.

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The rate of 20α-OHP accumulation ((pmol h^{-1} per 5×10^6 cells) during a 1-h incubation at 37 °C determined as described before, was 335.0 for thymocytes, 105.0 for spleen cells from nu/+ mice and <5.0 for spleen cells from athymic nu/nu mice. The results indicate that in lymphocytes the enzyme is only represented in the T-cell type.

It has been reported that thymic tissue and lymphocytes are capable of reversibly oxidising cortisol to cortisone[8], and that the activity of the enzyme that catalyses this oxidoreduction (11β-hydroxysteroid dehydrogenase) was 0.128 nmol per 100 mg tissue per 3 h incubation, which is $<1/1000$ of the activity of 20α-SDH reported here. 20α-SDH occurs in ovaries of many species and particularly in regressing corpora lutea of rodents, where its appearance signals the onset of luteolysis[9,10]. To our knowledge, no other enzyme activity connected with sex steroid metabolism has been reported to operate in the thymus.

During the incubation of radiolabelled progesterone with thymocytes we were able to detect a small amount of 5α-reductase activity, an enzyme which reduces progesterone to its 5α-pregnane derivative. The major pregnane product formed was identified as 5α-pregnane-3,20-dione by chromatography in 3 TLC systems, and by gas-chromatographic analysis of its *bis*-thioketal derivative[11]. In addition, the partially purified product was recrystallised with authentic 5α-pregnane-3,20-dione (Ikapharm) to constant specific activity.

The significance of the two progesterone metabolising enzyme activities in the thymus is not clear. Progesterone has been reported to be immunosuppressive[12,13] at high concentrations, which are achieved, probably, only near its site of formation in the foeto-placental unit. It is therefore conceivable that an enzyme engaged in reducing progesterone may have a role in the defence mechanisms mediated by thymocytes. Experiments are in progress to relate the distribution of 20α-SDH to that of known T-cell markers, such as θ antigen and responsiveness to plant lectins.

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Evidence for control of complement receptor rosette-forming cells by α- and β-adrenergic agents

PHARMACOLOGICAL agents such as dibutyryl cyclic AMP[1,2], cholera toxin[3,4] and β-adrenergic stimulants[5] have an effect on antibody production which can be inhibitory or enhancing, depending on the dose used and the time of addition of the agent. Such stimulants are known to inhibit the human T-lymphocyte receptor for sheep erythrocytes[6,7] and the murine lymphoma Fc receptor[8], and it has been shown that the complement receptor on B lymphocytes has an important role in the cellular events leading to antibody production[9,10], thus emphasising the need to clarify its nature. We report here that the number of complement receptor rosette-forming cells (EAC–RFC) in suspensions of mouse spleen cells can be decreased by α-adrenergic stimulants and increased by β-adrenergic stimulants, respectively.

Male CBA mice, of 3–5 months, were killed by ether inhalation. The spleens were teased in Eagle's minimal essential medium (MEM) to give a single cell suspension. The cells were washed twice and the concentration adjusted to 5×10^6 ml^{-1} in Eagle's MEM containing 10% heat-inactivated foetal calf serum incorporating various concentrations of the pharmacological agents under study. After 3-h incubation at 37 °C in a humid atmosphere containing 5% CO_2 in air, the cells were washed three times and 5×10^6 cells finally resuspended in 0.5 ml Eagle's MEM. The cell viability was judged to be 90–93% by the eosin dye exclusion test. For the estimation of complement rosettes, 0.5 ml aliquots of a 1% suspension of sheep red blood cells which had been freshly coated with rabbit anti-sheep red blood cell haemolysin (1: 1,000) and fresh mouse serum (1 : 20) as a source of complement, were centrifuged with an equal volume of the lymphocyte suspension at 200*g* for 2 min. The cell pellets were incubated together at 37 °C for 15 min, resuspended, and the number of rosette-forming cells counted. A rosette-forming cell was defined as a white cell with three or more adherent erythrocytes. Each study was done in duplicate and 400 cells were counted per tube. To identify the type of cells involved in the rosettes, 10 mM EDTA was added at the time of the rosette assays[11]. No significant change was observed. The rosette-forming cells in cultures of spleen cell suspensions incubated for 3 h with isoproterenol, adrenaline, noradrenaline, propranolol, dibutyryl cyclic AMP, dibutyryl cyclic GMP, aminophylline (all Sigma) or phentolamine (Regitin, CTBA) were therefore thought to be lymphocytes.

The effect on EAC–RFC of the β-adrenergic stimulator, isoproterenol, is shown in Fig. 1*a*. The number of EAC–RFC rose to a peak in a concentration of 5×10^{-5} M isoproterenol, and declined to almost to control values at 10^{-14} M. Isoproterenol is known to increase the concentration of intracellular cyclic AMP by stimulating adenyl cyclase activity[12]. The addition of dibutyryl cyclic AMP, however, had no effect on the number of EAC–RFC (Fig. 1*h*). When 10^{-4} M aminophylline, a phosphodiesterase inhibitor[13], was added with dibutyryl cyclic AMP, the number of EAC–RFC was unchanged (Table 1). The α-stimulant noradrenaline inhibited slightly the number of EAC–RFC. Adrenaline, which is thought to stimulate both the α- and β-adrenergic receptors, had no effect on the number of EAC–RFC (Fig. 1*e*). Presumably the effect of stimulating both receptors at the same time is to cancel out any effect on the number of EAC–RFC. It can be demonstrated, however, that adrenaline in such a situation is active, since its addition simultaneously with 10^{-5} M phentolamine (an α-blocker)[14] or 10^{-5} M propranolol (a β-blocker)[15] results in a significant increase or decrease, respectively, in the number of EAC–RFC (Fig. 1*f*,*g*). The concentrations of propranolol and phentolamine were such that neither drug on its own had a significant effect on EAC–RFC. It is probable that normal foetal calf serum contains a small quantity of adrenalines. Therefore the following experiment was done to clarify the effect of α-stimulant (noradrenaline) or β-stimulant (isoproterenol) in the foetal calf serum culture medium. When 10^{-5} M phentolamine was added to the medium containing isoproterenol, EAC–RFC increased in number as far as the concentration of the latter was

Table 1 Effect of pharmacological agents on complement rosette formation of mouse spleen cell suspensions at 37 °C for 3 h

	Mean % rosettes ±s.d.		Mean % rosettes ±s.d.
5×10^{-5} M isoproterenol (6)	33.80±2.96†	Control (C) (6)	22.74±1.81
10^{-15} M isoproterenol + 10^{-5} phentolamine (3)	32.91±1.80‡	C (3)	21.37±1.83
5×10^{-5} M noradrenaline (7)	15.35±1.90†	C (7)	23.22±1.70
10^{-6} M noradrenaline + 10^{-5} M propranolol (3)	8.16±1.80†	C (3)	21.08±0.52
10^{-6} M adrenaline (5)	23.10±0.98	C (5)	24.1 ±0.56
10^{-6} M adrenaline + 10^{-5} M propranolol (4)	11.31±2.30†	C (4)	20.75±1.85
10^{-5} M adrenaline + 10^{-5} M phentolamine (5)	30.10±3.02†	C (5)	21.05±0.73
10^{-3} M dibutyryl C-AMP (6)	21.30±2.00	C (6)	20.29±1.33
+ 10^{-5} M aminophylline (6)	20.54±0.44	C (6)	20.29±1.33

No. of duplicate cultures in brackets.
*Mean percentage rosettes = mean (no. of rosettes/no. of spleen cells) × 100.
†$P < 0.001$.
‡$P < 0.005$.
Statistical treatments were done with the difference between the mean value of rosette-forming cells of the test group and that of the control group.

kept between 10^{-11} M and 10^{-15} M (Fig. 1*b*). When 10^{-5} M propranolol was added to the medium in the presence of 10^{-4} M to 10^{-10} M noradrenaline, EAC–RFC decreased in number (Fig. 1*d*). Dibutyryl cyclic GMP seemed to have no effect on EAC–RFC (Fig. 1*i*).

Our results show that the number of EAC–RFC in a suspension of mouse spleen cells can be increased by the addition of a drug which stimulates the β-adrenergic receptor. Conversely, an agent which stimulates the α-receptor depresses the number of EAC–RFC. Further confirmation of these findings has been obtained from results which have shown that adrenaline (a stimulator of both α- and β-receptors) has no effect on the number of EAC–RFC in its own, but, with an α-blocker, adrenaline mimics a β-stimulator; when adrenaline and a β-blocker were used, the effect on the number of EAC–RFC is the same as that of an α-receptor stimulator (Fig. 1*f* and *g*).

α- and β-adrenergic receptors are thought to be regulatory (or receptor) units of the adenyl cyclase[16]. Activation of the β-receptor stimulates the enzyme and causes an increase in the level of intracellular cyclic AMP, whilst stimulation of the α-receptor causes a decrease in intracellular cyclic AMP.

We were, however, unable to demonstrate an effect on

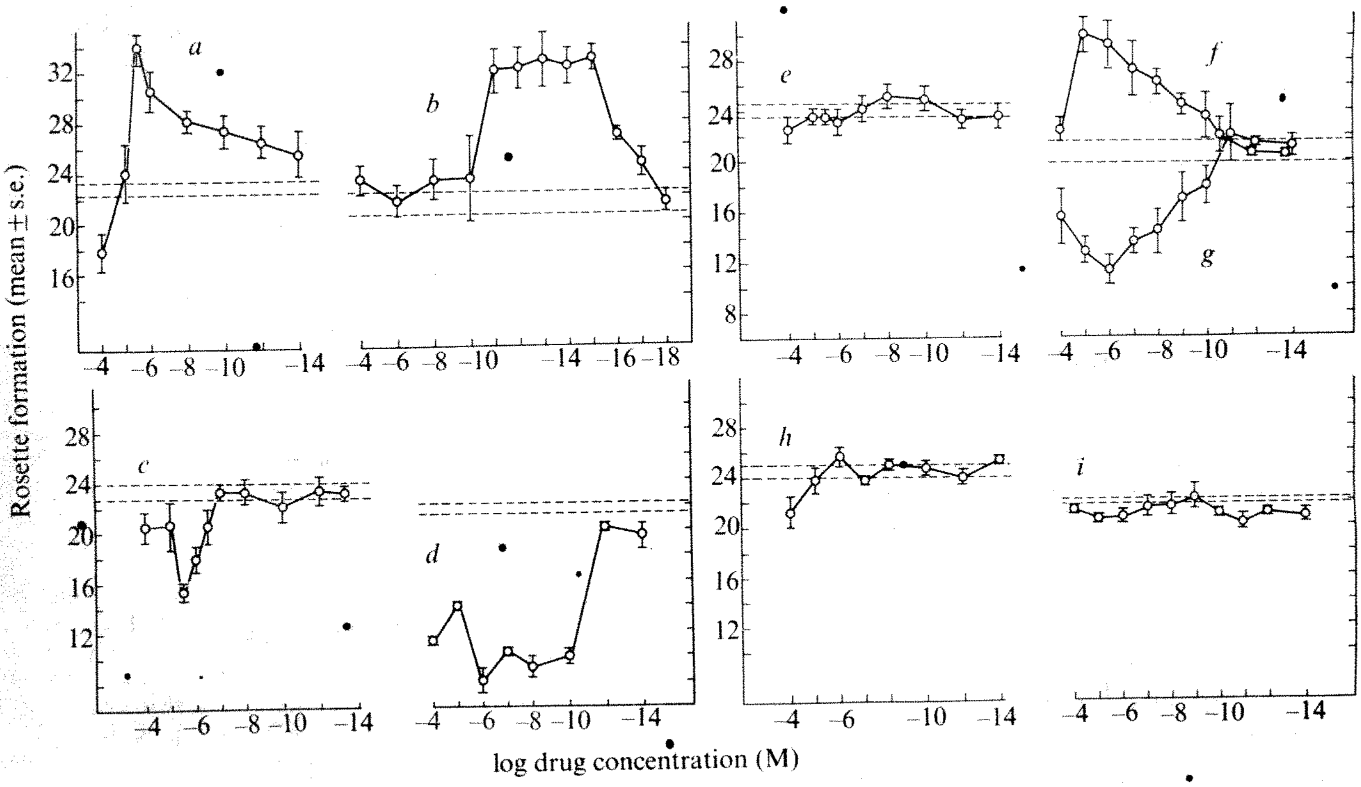

Fig. 1 Various concentrations of *a*, isoproterenol, *b*, isoproterenol + 10^{-5} M phentolamine, *c*, noradrenaline, *d*, noradrenaline + 10^{-5} M propranolol, *e*, adrenaline, *f*, adrenaline + 10^{-5} M phenatomine, *g*, adrenaline + 10^{-5} M propranolol, *h*, dibutyryl cyclic AMP, *i*, dibutyryl cyclic GMP were incubated with the mouse spleen cells at 5 + 10^6 cells per ml. Eagle's MEM (10% heat-inactivated foetal calf serum) in a 37 °C incubator in a 5% atmosphere for 3 h. After three washes EAC–RFC assay was performed as described in the text. Each point represents the mean rosettes, with ± s.e., of three to six animals. = = = =, ± s.e. of control of each group.

complement receptor rosettes of dibutyryl cyclic AMP, an agent believed to act beyond the level of adenyl cyclase. Furthermore, there was no effect when dibutyryl cyclic AMP was added with aminophylline, an inhibitor of phosphodiesterase, which suppresses the breakdown of intracellular cyclic AMP and thus effectively increases its intracellular concentration[13]. The rosette-forming cells, therefore, seem to be affected, not by the increase in the intracellular cyclic AMP levels, but by the increase in adenyl cyclase activity itself. It is known that B lymphocytes with complement receptor can produce antibody to T-dependent antigens[9,10]. On the other hand, catecholamines (adrenaline and noradrenaline) enter the blood stream, circulate throughout the body and affect various organs as hormones. We therefore suggest that in the immune system catecholamines influence the activity of complement receptor of B-lymphocyte, resulting in control of antibody formation.

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Response of mesencephalic and hypothalamic dopamine neurones to α-MSH: mediated by area postrema?

α-MELANOTROPIN (α-MSH) influences mammalian behaviour in a variety of situations. It is released from the pars intermedia of the pituitary during stress and may have a physiological role in adaptive processes[1,2]. In mammals, dopaminergic (DA) fibres of the tubero-hypophyseal system which originate in the arcuate nucleus of the hypothalamus, innervate the pars intermedia[3]; MSH secretion is usually inhibited by DA[4]. In rats, this DA system is subject to the influence of various extrahypothalamic brain regions thought to participate in the integration of endocrine and behavioural processes[5,6]. The tubero-hypophyseal DA neurones may therefore occupy a key position between brain areas involved in effects of behaviourally active peptide hormones on one hand and the pars intermedia, a site of production of such peptides, on the other hand. We report here that systemic injections of α-MSH in female rats affect the arcuate DA neurones as well as those of substantia nigra. The latter were studied in order to check for eventual parallel responses in an extrahypothalamic DA system. The peptide effect seems to depend upon the integrity of projections ascending from the lower brainstem. Cholinergic systems seem to participate in its manifestation.

We studied female SIV (Sprague–Dawley–Ivanovas) rats kept in groups of littermates under controlled illumination and temperature conditions (lights on 06.00–20.00; 22±1 °C). The animals were ovariectomised at the age of 4–5 months, 3 weeks before the experiments, and pretreated for one day with oestradiol-dipropionate (5 μg s.c.) and progesterone (2 mg s.c.), since we had used the same animal model in our studies on extrahypothalamic influences. The functional state of the DA neurone groups was assessed by a microfluorimetric procedure based on the histochemical fluorescence method of Falck and Hillarp. The distributions of fluorescence intensity of individual neurones are characteristic for a given functional state and neurone group. Studies on nigral DA neurones have revealed a close relation between mean intensity and mean firing rate; in agreement with earlier observations, a rise in mean intensity accompanied an increase in mean firing rate[7].

In the higher dose range which corresponds to that used in most behavioural experiments, α-MSH injected intraperitoneally (i.p.) (between 15.00 and 17.00) induced a marked rise in the mean fluorescence intensity of both DA neurone groups within 30 min (Fig. 1*a*, $P < 0.01$ for 100 μg per kg body weight; similar effect for 33 μg per kg but significant only in the arcuate nucleus). At lower doses, only the arcuate DA neurones responded ($P < 0.01$). This difference may have resulted from different sensitivities of the projection systems involved. The observation of a response of nigral DA neurones 15 min after injection of a lower dose in one rat suggests that the nigral reaction might also be more transient. If the relation established between cellular fluorescence intensity and neuronal activity[7] holds true also in the present case, the reaction of the arcuate DA neurones indicates an activation. Since DA inhibits MSH release[4], this corresponds to the activation of a negative feedback mechanism. The similar response of the nigral DA neurones may be linked with certain extrahypothalamic effects of the peptide. In analogy to the situation in the arcuate DA neurones, one may ask whether the nigral reaction might likewise reflect the activation of a compensatory mechanism. An aggravation of tremor by MSH has been noted in Parkinson patients[8]. Our findings are at variance with observations on repeated injections of α-MSH in male rats, where DA turnover in midbrain and striatum remained unchanged[9]. Whether this discrepancy is due to differences in the experimental arrangement or in the methods used for analysis of DA systems, cannot yet be decided. It seems conceivable that the hormonal state of the animal might influence peptide effects either qualitatively or quantitatively.

The response of the arcuate DA neurones cannot be expected to be specific for α-MSH, since this neurone group seems to be involved in the control of various pituitary hormones. Prolactin (secretion of which is likewise inhibited by this DA system) showed a similar effect (Fig. 1*b*, $P < 0.01$ compared with solvent- and to saline-injected groups). It can also be regarded as the activation of a negative feedback mechanism[10]. More surprising is the response of the nigral DA neurones ($P < 0.01$ versus solvent group, $P < 0.05$ versus saline group). It may reflect a direct effect of prolactin or be due to some more complex neuroendocrine mechanism. In contrast to what might have been expected in view of similarities between ACTH and MSH in some behavioural situations[1], comparable doses of $ACTH_{1-24}$ did not yield a clear-cut effect, but just a weak nigral reaction in half of the rats (Fig. 1*b*). The failure of $ACTH_{1-24}$ to affect the arcuate DA neurones is in keeping with the view that the DA system may be less important than noradrenergic neurones in the control of this hormone[11]. But changes in tubero-infundibular DA neurones have been

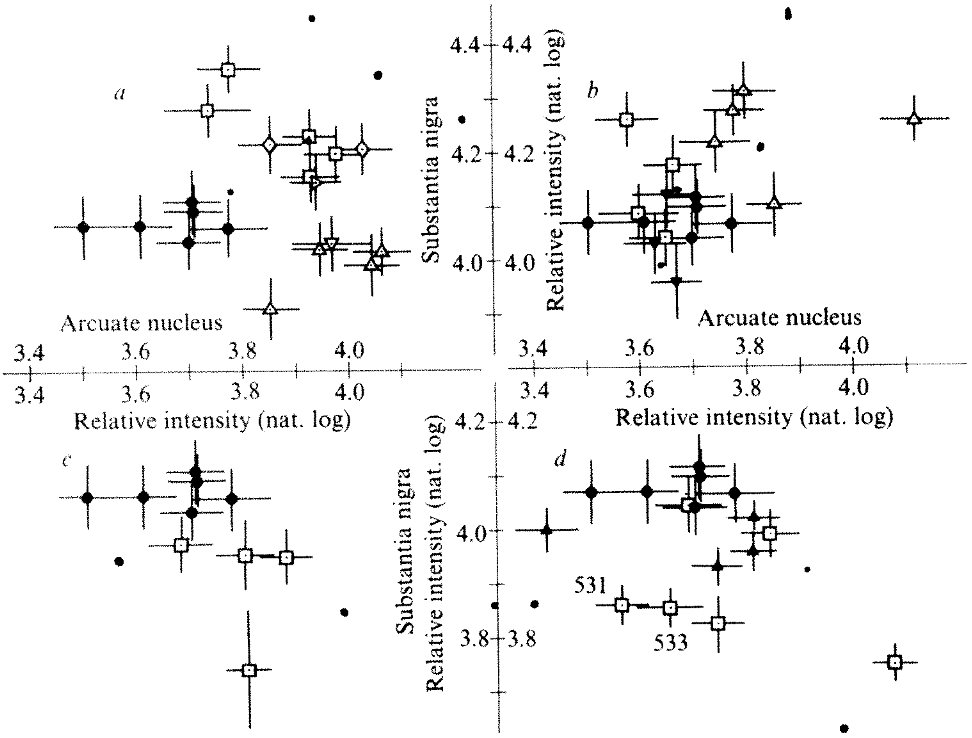

Fig. 1 Mean fluorescence intensity (bars = 95% confidence limits) of the DA neurone groups in the arcuate nucleus of the hypothalamus (abscissa) and in substantia nigra (ordinate) of individual rats. The saline-injected control group is shown for reference on each figure. *a*, Intensity 30 min (1 rat 15 min) after i.p. injection of α-MSH (Hoechst; dissolved in saline immediately before use, injection volume 0.1–0.2 ml per 100 g body wt.) at doses of (μg kg^{-1}) 100 (□), 33 (◇), 10 (△), 3 (▽), 10 (15 min; ▷) or saline (●). *b*, Effect of ovine prolactin 2mg per kg (Ferring; 1 mg per 0.25 ml solvent containing 1 mg phenol in 1 ml H_2O) (△) or $ACTH_{1-24}$ 175 μg per kg (Synacthen, CIBA; 0.25 mg ml^{-1}) (□) administered i.p. Doses are approximately equimolar to the highest dose of α-MSH used. Saline shown as (●); the phenol solvent itself remained without effect (▼). *c*, Blockade of the intensity change observed 30 min after α-MSH (100 μg kg^{-1}) by atropine 10 mg per kg injected 15 min before the peptide (□). Saline only (●). *d*, Mean fluorescence intensities in rats bearing lesions of area postrema as observed 30 min after i.p. injection of 100 μg per kg α-MSH (□) or saline (▲). Saline in non-lesioned animals (●). Numbers refer to the rats shown in Fig. 2. The values represent the combined right and left sides of the arcuate and nigral DA neurone groups; no differences were found between the two sides after lesions of area postrema. For each rat, the two brain pieces containing hypothalamus and midbrain were worked up together for microfluorimetry but investigated by different observers. The arcuate nucleus was studied at 15 antero-posterior levels (7-μm sections, distance between levels, 84 μm) starting at the anterior border of the external layer of the median eminence, the substantia nigra at 8 levels (distance between levels, 42 μm) starting at the anterior end of the fossa interpeduncularis[4,6]. In the former all visibly fluorescent catecholamine-containing cells were measured on both sides at each of these levels (mean 215±47 per rat), whereas in the latter 20 neighbouring cells per section were measured on one side starting at the dorsolateral corner of zona compacta (162±13 per rat, except for lesioned rats where both sides were studied: 279±41 per rat). The relative fluorescence intensity of a cell is defined by $i_r = (c-t)/(g_{NA}-g_t)$, c = absolute intensity of the cell, t = mean background fluorescence of neighbouring tissue, g_{NA} and g_t = mean intensities of noradrenaline-containing and noradrenaline-free standards of that section. For statistical analysis, the values were transformed into natural logarithms. Means of the transformed values are shown. Data were analysed by variance analysis followed by comparison of experimental groups with Scheffe's method of linear contrasts[4,5].

reported after manipulations of the pituitary adrenal axis[12]. Our results do not exclude an action of $ACTH_{1-24}$ on the two DA systems, but they indicate that this peptide was less effective than α-MSH in our experimental conditions.

The interpretation of central effects of peptides is complicated by the existence of the blood–brain barrier. Recent studies with labelled α-MSH suggest that the hormone may reach various brain regions, but its cellular localisation in brain tissue remains obscure[13]. The influence of α-MSH on extinction of a conditioned avoidance response is abolished by bilateral lesions in the parafascicular nuclei of the thalamus[14]. Although our observations need not be related to this particular aspect of MSH action, it may be noted that both DA neurone groups reacted to electrical stimulation in this nucleus and in neighbouring areas[6]. These observations, together with results of lesions in the dorsomedial tegmentum of the lower brainstem[6], seemed suggestive of a possible role of ascending projections. We focused our attention on the area postrema which is situated outside the blood-brain barrier[15]. Selective destruction of this area (Fig. 2) prevented the reaction of the arcuate DA neurones to α-MSH in four out of six rats (Fig. 1*d*, $P < 0.01$). In two rats with a partial response, we found intact tissue at the

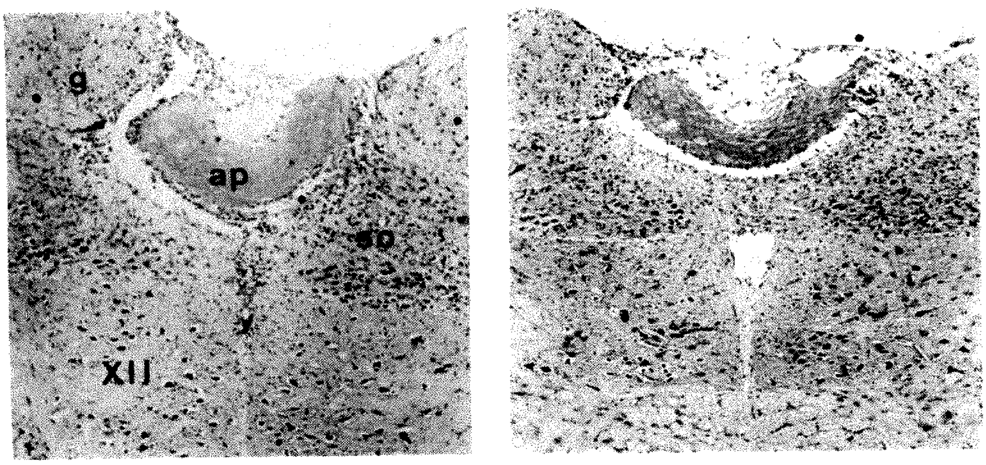

Fig. 2 Lesion of area postrema in rats nos 531 (left) and 533 (right). The lesion completely destroyed area postrema (ap) but impinged only slightly upon the dorsomedial corner of the nucleus of the solitary tract (so) and the medial edge of the nucleus gracilis (g). XII = nucleus nervi hypoglossi. Electrolytic lesions (250 μA, 60 s) were performed in gammabutyrolactone anaesthesia (750 mg kg^{-1} i.p., 1 h) according to coordinates calculated from various lesions in the lower brainstem, 2 weeks after ovariectomy. α-MSH was injected 5 d later between 15.00 and 16.00, after the usual 1-d steriod pretreatment. The rats recovered well from the operation and showed no neurological disturbances. They lost 6% of body weight, as did animals with ineffective electrode placements (×49.2).

posterior and anterior extremities of area postrema, respectively. In all rats, the reaction of the nigral DA neurones was not only blocked but apparently reversed, as mean intensity decreased to below the level of saline-injected intact rats ($P < 0.01$; saline-injected lesioned rats exhibited intermediary levels). Rats with electrode placements in the fourth ventricle in front of area postrema resulting only in a slight superficial damage to the ventricular wall, exhibited the usual reaction to α-MSH (mean intensity ±S.D. of these 3 rats (arcuate–nigral DA group): 3.965±0.3966 ($n = 202$)/4.386±0.3191 ($n = 240$); 3.837±0.4470 (140)/4.282±0.3570 (241); 3.987±0.6252 (103)/ 4.354±0.3561 (240).)

The effect of α-MSH thus seems to depend on the integrity of area postrema. It seems conceivable that α-MSH acted on this area, but a modulatory action of the peptide exerted at another brain level on an influence arising from area postrema, cannot be ruled out. The observations on the nigral DA neurones indicate that the effect of the lesion may be a complex one. The projection systems involved remain to be characterised. As atropine blocks responses of both DA neurone groups to electrical stimulation in various extrahypothalamic areas[5,6], we investigated its effect (Fig. 1*c*). Atropine completely prevented the intensity change in the nigral DA neurones ($P < 0.01$ against MSH without atropine). In the arcuate nucleus, the response was only partially reduced. Thus, cholinergic systems seem to be involved in the peptide effect, but the circuitry may differ in detail with respect to the two DA neurone groups.

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Possible relationship between glial cells, dopamine and the effects of antipsychotic drugs

THE hypothesis that a functional excess of dopamine underlies many of the abnormalities seen in schizophrenia is receiving much attention. The supporting evidence is principally pharmacological. There is a correlation between antipsychotic drug action and the ability of a neuroleptic drug to block dopaminergic transmission[1–3]. The exact site of antipsychotic drug activity is not known and evidence for the presynaptic inhibition of dopamine release[4], as well as the blockade of postsynaptic receptors[5,6] has been presented to explain neuroleptic drug action. Studies of binding properties of the dopamine receptor, however, provide a coherent explanation of neuroleptic drug action. Butyrophenones, phenothiazines, thioxanines and diphenylbutylpiperadines inhibit ^{3}H-haloperidol binding to a central nervous system (CNS) membrane fraction in exactly the order of their clinical potency[7–10]: the inhibition occurs at drug concentrations similar to those expected in the CNS of patients taking these drugs. These results with ^{3}H-haloperidol, a dopamine antagonist, are used to argue that neuroleptics bind to postsynaptic dopamine receptors, and are effective by decreasing the activity of pathways using dopamine as their neurotransmitter. On the other hand, dopamine-sensitive adenylate cyclase is associated with the postsynaptic receptors[5,11], yet the inhibition of this enzyme by neuroleptic butyrophenones does not correlate with *in vivo* or clinical potency. The difference between butyrophenone inhibition of ^{3}H-haloperidol binding and inhibition of adenylate cyclase has been attributed to "variable degrees of coupling of dopamine receptor sites with the adenylate cyclase"[9]. Alternatively, there could be several dopamine sensitive adenylate cyclases with differing binding affinities and localisations: this is suggested by the presence of this enzyme in several tissue culture lines of glia[12]. Lesion studies show that the enzyme is not localised presynaptically in the caudate nucleus[13] or in the substantia nigra[14]. The only defined dopaminergic receptors in the substantia nigra are localised on dopaminergic neurones[15], and the elimination of these cells as the site of the enzyme was established by unilateral injections of 6-hydroxydopamine which removed these cells[14]. It therefore seems necessary to define more exactly the site of the dopamine binding receptor and the dopamine-stimulated adenylate cyclase. We report here that a large proportion of CNS dopamine haloperidol binding sites seem to be present on glial membranes and may be associated with an adenylate cyclase localised on these membranes.

We undertook to localise dopamine and haloperidol binding by fractionating rabbit cortex and bovine caudate and cortex. Synaptic fractions were prepared by the method of Gray and Whittaker[16], and the P_2 pellet of this preparation was used as a cell-free CNS membrane fraction. Glial cell fractions were prepared[17] and a C-6 cultured glial cell line was used for testing a clonal glial cell line. Binding assays were carried out in the presence of D and L butaclamol. Only the D isomer is active pharmacologically in blocking dopamine binding or as a clinical neuroleptic. Thus the difference between labelled dopamine and haloperidol binding in presence of the two isomers is taken as a measure of the blockade of specific dopamine receptors[7–10]. The assay used was essentially the centrifugation assay of Seeman *et al.*[8]. Although the filtration assay used by these workers and Snyder's group[7,9] seemed to give similar results, we did not achieve the precision using this assay that was achieved using centrifugation and ^{14}C-inulin to account for trapped external media in the pellet. Assay conditions were as in ref. 8 except that a different monoamine oxidase inhibitor (10.5 μM pargyline-HCl) was added to buffer solutions.

Our initial results with fractions isolated from rabbit cortex showed some purification of dopamine binding in the membrane fraction (P_2 pellet) derived from CNS homogenate, but little subsequent purification when a synaptosomal fraction was isolated. This prompted us to look at the binding of ^{3}H-dopamine and ^{3}H-haloperidol on fractions isolated from beef caudate. The results in Table 1 clearly show that although there is some purification of the receptor in isolating the P_2 pellet, little further enrichment occurs when this fraction is subjected to density gradient centrifugation to isolate synaptosomes. After obtaining these results fractions enriched in astroglia were obtained from bovine caudate. An excellent glial cell fraction was isolated using the method for rabbit cortex with a slight modification

Table 1 Binding of ^{3}H-dopamine and ^{3}H-haloperidol, and activity of glutamic acid decarboxylase (GAD) in fractions isolated from the caudate nucleus of bovine brains

	Dopamine binding Δfmol per mg protein	Haloperidol binding Δfmol per mg protein	GAD activity μmol per mg per h
Homogenate	9.25 ± 3.4	3.9 ± 1.1	28.6
P_2 fraction	17.65 ± 3.7	3.8 ± 0.9	54.8
Synaptosomal fraction	21.00 ± 3.5	6.5 ± 1.3	59.8
Glial fraction	35.05 ± 0.7	16.1 ± 0.9	4.0

Dopamine binding was measured with a final concentration of 1 nM ^{3}H-dopamine in the presence of 1 μM (+) or (−) butaclamol. Haloperidol binding was measured with 1 nM ^{3}H-haloperidol in the presence of 0.1 μM (+) or (−) butaclamol. The difference in binding between (+) butaclamol, a potent neuroleptic drug, and (−) butaclamol, an inactive drug, is taken as the stereospecific binding of the ligand. Binding assays represent triplicate determinations on at least two different glial cell preparations.

in the final Ficoll gradient[17]. The glia were collected between Ficoll solutions having refractive indices of 1.3633 and 1.3714, respectively. Binding studies in homogenates of these glial cells revealed a considerable purification of the receptor. This unexpected result suggests that the receptor which mediates neuroleptic drug activity is localised on glial membranes.

To assure that both dopamine and haloperidol binding take place on glial membranes we carried out several checks on the purity of our fractions. Morphological investigation by light and electron microscopy suggested that approximately 60% of the synaptosomal pellet is synaptic material, consistent with our previous findings on synaptic fraction purity[18]. The glial fractions also contain appreciable cell debris and, when viewed in the light microscope, 20% of the material seemed to be of non-glial origin. To quantitate this we assayed glutamic acid decarboxylase (GAD), the γ-aminobutyric acid (GABA) synthesising enzyme, in our fractions. The caudate is rich in GABA[19] and GAD has been found almost exclusively in synaptic regions[20]. The enzyme was assayed by the method of Roberts and Simonsen[21]. The results suggest that there is enrichment of synaptic material containing GAD in both P_2 and synaptosomal fractions while there is almost no GAD present in the glial fractions (Table 1). In addition, dopamine or haloperidol binding activity was found in a homogenate of C-6 glial cells, cells originating from a cloned line of rat glioma cells[22]. Furthermore, the glial dopamine-binding receptor seemed to be localised to regions of brain rich in dopamine in preliminary experiments.

To examine the possibility that glial dopamine sensitive adenylate cyclase could be associated with the binding site measured in either the ^{3}H-dopamine or ^{3}H-haloperidol assay, we examined the purification of the enzyme during cellular and subcellular fractionation. Cyclic 3′-5′ AMP was measured in fractions using a modification of the protein binding assay of Gilman[23] (from Amersham Searle). The difference between dopamine-stimulated activity and basal activity was taken as a measure of the dopamine sensitive enzyme. The results of measurements on fractions from bovine caudate show that the enzyme activity is purified to approximately the same extent in glial cells as the binding site for ^{3}H-dopamine or ^{3}H-haloperidol. The enzyme showed 3.8-fold higher activity in the glial fraction than in the crude homogenate, and the binding of dopamine and haloperidol are similarly increased by fourfold. This finding, coupled with the results of Schubert *et al.*[12] demonstrating that clonal lines of glia contain a dopamine-stimulated adenylate cyclase and that in one glial line this enzyme was inhibited by haloperidol, suggests that the receptor in caudate could be an adenylate cyclase localised in glial cells.

The finding of a dopamine binding site on glial cells which may be associated with an adenylate cyclase suggests that a mechanism exists which would cause an increase in cyclic AMP in glia in response to neronal activity. Such a metabolic response to neural release of neurotransmitter provides the initial step in what could be an important feedback control regulating neuronal activity. The morphology of the synapse is consistent with such a control system, for synapses are surrounded by an astroglial sheath[24]. Such a control system could involve changes mediated by cyclic AMP analogous to the increased glycogen breakdown seen in skeletal muscle[25] or membrane permeability changes as seen in smooth muscle[26], both mediated by a catecholamine-sensitive β-adrenergic receptor. Such an intercellular control provides a mechanism through which neuroleptic drugs could have a regionalised effect on CNS activity and dampen a wide variety of functions. This is an interesting possibility in view of the variety of symptoms seen in psychotic illness and the fact that neuroleptic drugs are neither specific nor curative in schizophrenia. The results of this preliminary study thus suggest that the receptor which correlates with the site of neuroleptic drug binding may reside on glial membranes and thus point to a possible mediation of neuronal activity by glial cells.

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Human prolactin-responses to neuroleptic drugs correlate with antischizophrenic potency

THE secretion of prolactin, a pituitary hormone, is regulated by inhibitory and stimulatory influences from the hypothalamus. The primary influence is tonic inhibitory[1]. Dopamine, a neurotransmitter, has a major direct and/or indirect role in mediating this inhibition upon the prolactin cells in the pituitary gland. This is documented by studies *in vitro*[1], *in vivo*[2] and in man[3]. All known neuroleptic, antischizophrenic drugs share one characteristic—they block dopaminergic transmission[4–7]. This action is hypothesised to be associated with their antischizophrenic effects (dopamine hypothesis)[8,9]. Dopaminergic blockade is, very likely, also the primary mechanism of neuroleptics when inducing prolactin release via disinhibition[1,10]. There is no evidence that neuroleptics release prolactin via stimulatory influences. We believe the prolactin response to neuroleptic drugs to be a valid and reliable model of dopaminergic blockade in man (Langer *et al.*, unpublished). Intesting the dopamine hypothesis of antischizophrenic drug action in man we postulated that comparing drug effects after acute intramuscular administration, a high correlation between prolactin releasing and antischizophrenic potencies of neuroleptics would be found.

Twelve healthy young male volunteers (medical students) were screened medically and psychiatrically for participation in the study and informed consent was obtained. On an average, each subject participated in seven weekly sessions, each starting at about 9 a.m., after an overnight fast, and lasting for 3½ h. At each session a single drug and dose was intramuscularly (i.m.) administered 30 min after a small angiocath had been inserted into a forearm vein of the recumbent subject. Throughout the study, blood samples were withdrawn at 15-min intervals into heparinised tubes. The plasma was immediately separated after the study and frozen at –20 °C. Prolactin was analysed in duplicate by radioimmunossay[11]. All samples from each subject were determined in one assay; variation between duplicate samples was 5.3%.

The following antischizophrenic drugs were selected because of their wide use in clinical psychiatry (which results in adequate documentation of their antischizophrenic effects) and because they are all intramuscularly administerable: haloperidol (0.25, 0.5, 1.0 and 1.5 mg), chlorpromazine (25.0 mg), prochlorperazine (2.5 and 5.0 mg), trifluoperazine (4.0 mg), perphenazine (1.0 mg), fluphenazine (0.35 mg), and thiothixene (0.25 and 0.5 mg). There is considerable interindividual variation of the prolactin response to a neuroleptic drug. We minimised the variation by (1) testing several drugs within the same subject, and (2) relating each subject's peak prolactin response to a neuroleptic to his own haloperidol dose–prolactin response curve. Haloperidol, a relatively specific dopamine receptor blocker and potent antischizophrenic drug, was chosen as reference drug for prolactin releasing and clinical potencies. So, all subjects were first tested with usually four different doses of haloperidol. Five of the subjects were then tested with two different doses of prochlorperazine and two different doses of thiothixene. We demonstrated that the dose–prolactin response curves to haloperidol, prochlorperazine and thiothixene, representing three different chemical classes of neuroleptics, were parallel (Langer *et al.*, in preparation). Eight of the subjects then received single doses of the various neuroleptics in doses which, according to a pilot study, induced about 50% of maximal prolactin release. To evaluate relative potencies, the peak prolactin response to a drug was related to the individual haloperidol dose–prolactin response curve. The potency per mg of each drug in releasing prolactin was then calculated in per cent potency of haloperidol in releasing prolactin. Finally, we related this value to the clinical potency of each drug relative to haloperidol. Data on antischizophrenic potencies of drugs after acute parental administration were derived from standard clinical references[12,13].

Our results (Fig. 1) show that seven widely used antischizophrenic drugs show a very high correlation ($r = 0.956$) between their potency to release prolactin in man and their potency in the acute treatment of schizophrenic patients.

We thus hypothesise that mechanisms mediating these two different drug effects have at least one critical variable in common—the blockade of dopaminergic transmission. The parallelism of dose–prolactin response curves of the three representative drugs also indicates a common, dopaminergic, mechanism. We limit the interpretation of our findings to the seven antischizophrenic drugs we tested, which constitute the majority of drugs used in the treatment of schizophrenia. So far, only one antischizophrenic, antidopaminergic drug (clozapine) shows inadequate prolactin-releasing activity. This might indicate that (1) increase of prolactin is not necessary for therapeutic response, and (2) different dopaminergic systems are primarily involved in prolactin-releasing and antischizophrenic effects. Prolactin release by neuroleptics probably reflects blockade of dopaminergic transmission at the hypothalamic and pituitary level, rather than at extrahypothalamic (possibly limbic) loci which are hypothesised to be associated with the therapeutic actions of antischizophrenic drugs[14]. It is noteworthy that schizophrenic patients generally require treatment of higher doses of a neuroleptic than are needed to achieve maximal prolactin release (1–2 mg haloperidol i.m. per 70 kg or dose equivalence of another neuroleptic drug).

This study supports in man the hypothesis that blockade of dopaminergic transmission is a characteristic of the neuroleptic drugs we tested and associated with their antischizophrenic effects. The questions whether the pathogenesis of schizophrenia specifically involves a disturbance in brain dopaminergic function and whether drugs necessarily have to interfere with

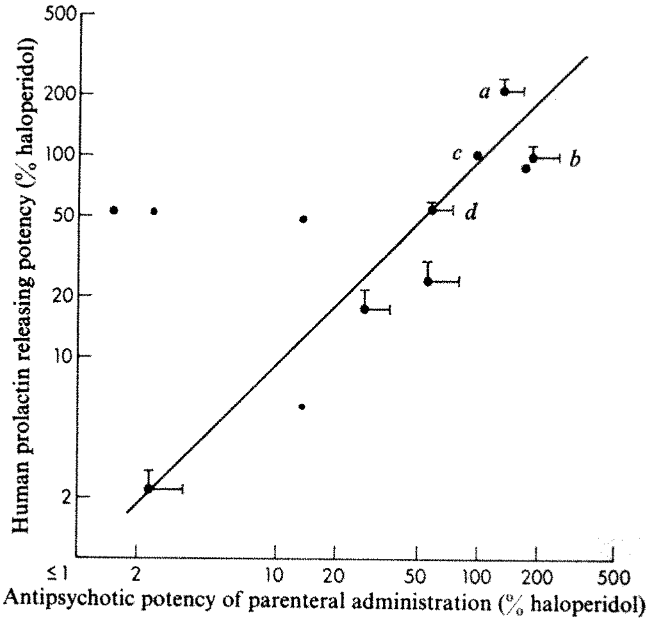

Fig. 1 The potency of seven antischizophrenic drugs in inducing prolactin release in man correlates with their potency in treating schizophrenic patients. Drugs tested (number of subjects in parentheses): *a*, thiothixene (6); *b*, fluphenazine (5); *c*, haloperidol (12); *d*, perphenazine (6); *e*, trifluoperazine (7); *f*, prochlorperazine (7) *g*, chlorpromazine (8). All values refer to an acute intramuscular administration and are expressed in per cent potency of haloperidol. Each value is the mean; vertical bars show the s.e.m. of prolactin values, horizontal bars the range of potency with conventional treatment. $y = 0.212 + 0.93x$, $r = 0.956$.

dopaminergic transmission to achieve antischizophrenic effects await the elucidation of the biological basis of schizophrenia.

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Defective dopaminergic regulation of prolactin secretion in a rat pituitary tumour cell line

IT is firmly established that certain rat[1,2] and human[3,4] pituitary tumours hypersecrete prolactin (PRL). Pituitary PRL secretion is probably under the dual control of hypothalamic prolactin-inhibiting and -releasing factors, and defective secretion in one or both of these factors could produce abnormal PRL secretion[3]. An alternative explanation for the *in vivo* PRL secretory defect of certain pituitary tumours is defective reception by the tumour of an appropriate hypothalamic endocrine signal. To test this latter hypothesis, we evaluated the effects of various catecholamines on PRL secretion in a cloned rat pituitary tumour cell line (GH_3). Catecholamines are potent inhibitors of PRL secretion[5], and it is postulated that the prolactin inhibiting factor may be a catecholamine[6,7]. We report here that dopamine failed to inhibit PRL secretion from the pituitary tumour cultures, whereas, other catechols added to our normal and pituitary tumour monolayer cultures suppressed prolactin release into the medium. In addition, apomorphine, which is postulated to be a specific dopaminergic agonist[8–10], appropriately suppressed PRL secretion in the tumour monolayer cultures. Our results therefore provide evidence for an unusual dopaminergic defect in the GH_3 rat pituitary tumour cell line.

The pituitary tumour cell line was acquired from ATCC and was subcultured according to the method of Sato and Tasjian[11]. Our normal rat pituitary monolayer cultures were produced by a modification of the method of Vale *et al.*[12] (Fig. 1). Rat PRL was measured by radioimmunoassay[13].

Figure 1 illustrates that dopamine not only failed to suppress PRL secretion, but paradoxically stimulated PRL release in the pituitary tumour cell cultures. Significant PRL stimulation, however, was observed in most but not all of the individual experiments. Conversely, dopamine produced a significant suppression of PRL secretion in the preconfluent normal rat monolayers. Other catecholamines added to the GH_3 cells and the normal rat pituitary cultures also produced suppression of PRL secretion.

The incubation conditions of our monolayer cultures for both the tumour and normal pituitary cells were similar (see Fig. 1), except that 15% horse and 2.5% foetal bovine serum were used with the tumour cells, and 20% foetal bovine serum was used in the normal rat monolayers. Dopamine is known to be susceptible to oxidant stress[14] and we therefore had to demonstrate that the failure of dopamine to inhibit PRL secretion in the tumour cultures was not secondary to its rapid destruction by the horse serum. When dopamine was added to the normal pituitary cultures with 15% horse serum and 2.5% foetal bovine serum, PRL secretion was significantly inhibited, whereas dopamine added to the tumour cultures with 20% foetal bovine serum still failed to inhibit PRL secretion. Hence, failure of dopamine to inhibit prolactin secretion in the pituitary tumour monolayer cultures was not secondary to rapid destruction by the horse serum.

Another possible explanation for the lack of dopamine inhibition of PRL secretion was that it was a dose-related event. It has been suggested that adrenaline at low doses *in vitro* produced stimulation, and at high doses inhibition of PRL secretion[15]. To demonstrate that a biphasic effect was not present in the tumour cultures, PRL secretion was evaluated after addition of various concentrations of dopamine over a 40-fold range. In this experiment, each concentration of dopamine failed to significantly alter PRL secretion in the pituitary tumour cultures, whereas there was progressive inhibition of PRL secretion in the normal cultures of 25% by 1.5×10^{-6} M, 65% by 1.5×10^{-5} M, and 80% by 6×10^{-5} M dopamine. Hence, the PRL secretory defect was not explainable on the basis of the concentration of dopamine used in the experiments and was consistent with the presence of a dopaminergic defect in this pituitary tumour cell line. The observation, however, that apomorphine significantly suppressed PRL secretion in the tumour cultures (Fig. 1, Table 1) was difficult to reconcile with this conclusion. Apomorphine has been shown to be a dopaminergic agonist[8–10] and the ability of pimozide, a specific dopaminergic blocking agent[16–18] to inhibit the suppressive effect of apomorphine on PRL secretion in the tumour cultures (Table 1) suggested that certain aspects of dopaminergic function were intact in the tumour cultures. Recent evidence has suggested that apomorphine may act only at presynaptic dopaminergic sites[19], however, this finding does not explain our observations, as dopamine probably acts at both pre- and postsynaptic receptors[19]. It is possible therefore that the tumour cell line may have acquired a dopaminergic defect that permitted suppression of PRL secretion with apomorphine and yet failed to respond in a similar manner to dopamine. Alternatively, our data suggest that dopamine and apomorphine act at different sites in the rat pituitary. Hence defective dopaminergic regulation of prolactin secretion exists in this rat pituitary tumour cell line and may be related to the excessive prolactin secretion exhibited by certain rodent and human pituitary tumours.

Table 1 The effect of a dopamine agonist and antagonist on pituitary tumour prolactin secretion.

Treatment	n	Rat PRL (μg per well) 0 h	Rat PRL (μg per well) 6 h
Controls	4	1.40±0.09	1.89±0.12
APO	5	1.16±0.19	1.37±0.11*
PIM	5	1.38±0.09	1.79±0.10
APO+PIM	5	1.53±0.06	1.92±0.04

Data are means±s.e.m. APO, apomorphine 2.5×10^{-5} M; PIM, pimozide 4.0×10^{-6} M.
*$P<0.02$.

This latter postulate, however, has to be viewed in the light of a large number of reports that indicate that L-dopa and/or its metabolite, dopamine, can inhibit rodent[2] and human[3,4,20]

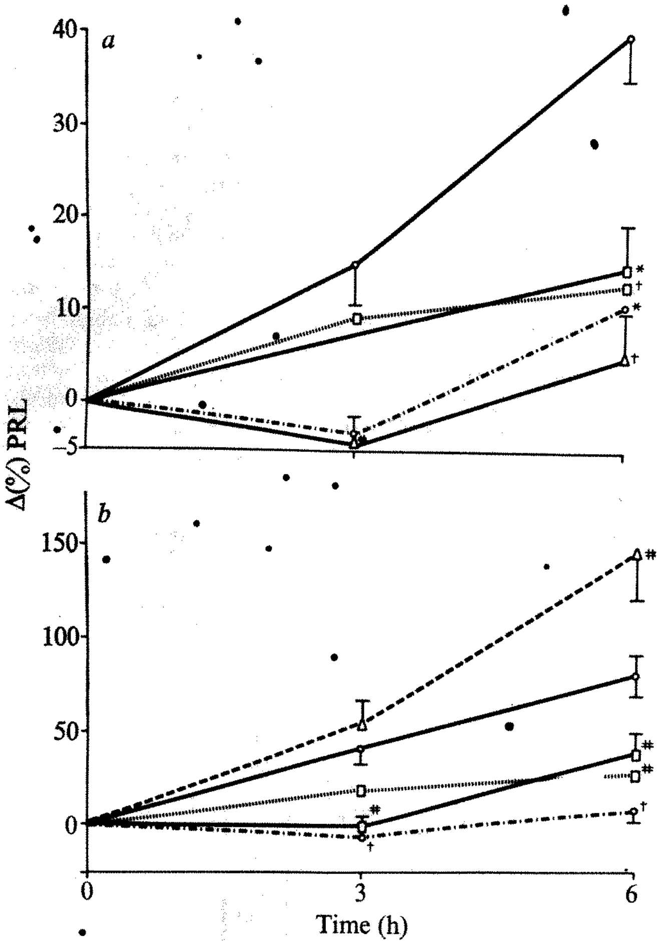

Fig. 1 The effect of catecholamines on PRL secretion in *a*, normal and *b*, tumour-bearing rat pituitary monolayer cultures. Normal cultures were prepared from anterior pituitaries removed from Holtzman male rats (200–300 g) immediately after killing by guillotine. Tissues were minced into 2-mm pieces with a sterile scalpel, and incubated for 45 min with gentle stirring at 37 °C with 0.5% collagenase (CIS Worthington) in 1X Detroit MEM with HEPES buffer at *p*H 7.2, supplemented with gentamycin (50 μg ml^{-1}), 1X amino acids (1 ml per 100 ml), 1 mM sodium pyruvate (1 ml per 100 ml), and 20% foetal calf serum (Grand Island Biochem.). The cell suspension was then centrifuged at 650*g* for 7 min. The supernatant was removed and the pellet resuspended with 10 ml of medium. This centrifugation and pellet resuspension in fresh medium was repeated once, and the final suspension was placed in 60×15 mm Linbro plates at a concentration of 3×10^4 to 7×10^4 cells per well to a final volume of 4 ml per well. All incubations were carried out at 37 °C (95% room air, 5% CO_2). The growth medium on these pre-confluent cultures was changed every 2–3 d and 10–24 h before the beginning of each experiment. At the beginning of an experiment, a 200-μl aliquot of the medium was removed from each well and stored at −20 °C for radioimmunoassay. The test substances were then added to each well and the progress of the experiments was followed by taking additional samples of the medium at 3 and 6 h. Rat PRL was measured by radioimmunoassay using RP-1 obtained from the NIAMDD as standard with tubes added to the assay to control for serum effects produced by the tissue culture medium. All of the catecholamines were prepared in the dark on ice immediately before addition to the culture to minimise oxidation. GH_3 cells (passage 20) were subcultured with trypsin (0.01 M) according to the methods of Sato and Tashjian[5], and seeded in 60×15-mm Linbro plates at a concentration of 2×10^5 to 4×10^5 cells per well to a final volume of 4 ml per well. The culture medium consisted of Hams F-10 medium, supplemented with 2.5% foetal bovine serum, and 15% gamma-globulin-free horse serum (Grand Island Biochem.) and the *p*H was adjusted to *p*H 7.2. These pre-confluent cultures were allowed to grow for at least 72 h before study. After an experiment PRL secretion in each monolayer culture returned to its basal secretory rate. The normal and tumour figures represent pooled data from 8 and 9 separate experiments, respectively. PRL concentrations are expressed as % change from the basal PRL level. The data was transformed because of variations in basal PRL concentration of the individual wells between experiments and occasionally within the same experiment. Vertical bars represent s.e.m. and Student's *t* test was used to evaluate the means with a P of < 0.05 being considered significant. △ Represents results for dopamine 4×10^{-5} M ($n = 25$ in *a*; 34 in *b*); ○ (on solid line) controls ($n = 41$ in *a*, 50 in *b*); ○ (broken line), adrenaline 4.5×10^{-5} M ($n = 5$ in *a*, 9 in *b*); □ (solid line), apomorphine 2.5×10^{-5} M ($n = 9$ in *a*, 13 in *b*); □ (broken line), noradrenaline 4.5×10^{-5} M ($n = 13$ in *a*, 11 in *b*); significance levels shown as *, $P < 0.05$; ‡, $P < 0.002$; †, $P < 0.002$.

pituitary tumour PRL secretion. Interpretation of this *in vivo* data is complicated, however, by the observation that α adrenergic blockade can inhibit the effect of L-dopa in decreasing rat pituitary tumour PRL secretion[2]. In addition, a recent report by Van Der Gugten *et al.*[21] suggests that L-dopa decreased serum PRL concentrations in the rat in part by stimulating the peripheral uptake of PRL. Thus the reported suppressive effects of L-dopa on pituitary tumour PRL secretion may not be exerted via the dopaminergic receptor. Whether the dopaminergic defect described here is unique to this cloned cell line, or reflects a generalised defect in PRL secreting pituitary neoplasms remains to be established.

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Prolactin–progesterone antagonism in self regulation of prolactin receptors in the mammary gland

THE role of prolactin during growth of the mammary gland and in lactation is well established (see ref. 1 for review) and the antagonising action of progesterone on lactogenesis (but not on mammary growth) has been recognised[2–4]. A specific receptor for prolactin has been described[5] and purified[6] and it seems that the level of this receptor in the target cells is very sensitive to hormonal environments and might be one of the essential parameters which modulates the intensity of prolactin action. Titration of this receptor under controlled hormonal conditions has been mainly performed in the liver and its concentrations found to be sensitive to oestrogens[7]; this effect may be amplified or even mediated by prolactin itself[8]. We have shown[9] that the number of prolactin receptors in the mammary gland of pregnant or lactating rabbits undergoes a sudden increase at the onset of lactation but that the K_a of the receptor–hormone interaction remains constant. The question arises as to which hormonal environment is required for this amplification; is the inhibitory action of high progesterone levels on lactogenesis associated with a reduction of receptor concentrations? We describe here a study of this possibility and of the positive regulation of prolactin on its own receptors in the rabbit mammary gland. We extend to the mammary gland the stimulating effect of prolactin on the levels of its receptors and demonstrate an antagonising action of progesterone on this process.

New Zealand rabbits (4–6 months old) were used either in pseudopregnancy (mating with a vasectomised male) or at late pregnancy. Pseudopregnant animals were treated intramuscularly (i.m.) with 400 or 50 IU ovine prolactin (Byla) divided into four injections (two per d) over 48 h. Progesterone (Roussel) when administered was injected i.m. at a daily dose of 10 mg (two times 5 mg), starting the day before prolactin treatment. *In vivo* desaturation of receptor sites of their endogenous prolactin with bromocriptine (CB 154, Sandoz) was performed by injecting the drug subcutaneously (2 mg) 36, 24 and 12 h before the animals were killed, the first injection taking place 12 h after the last prolactin (and progesterone) treatment. Receptors were assayed as described before[9] by estimating their total amount and their affinity constant according to the Scatchard plots[10] obtained with varying concentrations of ovine prolactin labelled with ^{125}I and substracting the non-specific binding. Protein concentrations were assayed according to Lowry *et al.*[11] and DNA according to Morimoto *et al.*[12].

Figure 1 shows that the total number of receptors per mg DNA in the mammary gland increases strikingly at parturition as shown previously[9], but that this increase is inhibited by 60% ($P < 0.01$) when the prolactin surge at parturition is blocked by injecting CB 154 to the animals on the last day of pregnancy. In both cases, prolactin receptors were measured 6 d after parturition, since this delay corresponds to the highest levels normally observed. Interestingly the reduction in prolactin receptors was associated with a significant reduction in milk production (results not shown). This experiment is an illustration of the prominent role of prolactin inducing its own receptor at parturition, although it must be emphasised that this role probably requires a definite hormonal environment, since prolactin levels remain high in the rabbit at the onset of lactation, but seem unable at this period to act strongly on receptor concentrations. Figure 2 shows the effect of four doses of 12.5 IU and four of 100 IU of prolactin on the pseudopregnant rabbit and demonstrates the ability of the higher dose of this hormone to increase its receptors in the mammary gland. The lower dose, though inactive when injected during 48 h, becomes effective when administered through more extended periods (results not shown). Progesterone injected simultaneously with a total dose of 400 IU of prolactin completely prevents any increase of the prolactin receptors in the mammary gland. This result extends the classical inhibitory effect of

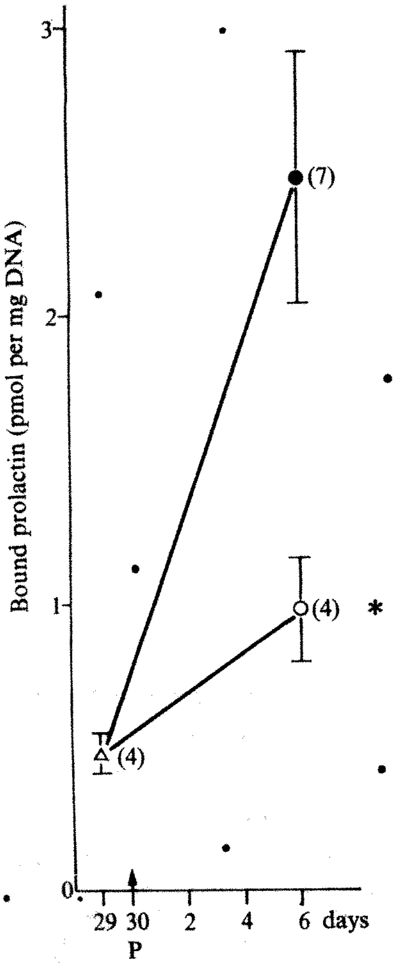

Fig. 1 Total number of prolactin receptors in the rabbit mammary gland titrated after 36 h *in vivo* desaturation. In the following conditions. △, Day 29 of pregnancy; ●, day 6 postpartum; ○, day 6 postpartum, prolactin surge at birth suppressed by ergocryptine (see text). Each point represents the mean±s.e. for the number of animals indicated in parentheses. The affinity constant of the hormone–receptor interaction did not vary ($K_a = 3 \times 10^9\ \mathrm{M}^{-1}$). *Significantly lower than control at the same day ($P < 0.01$).

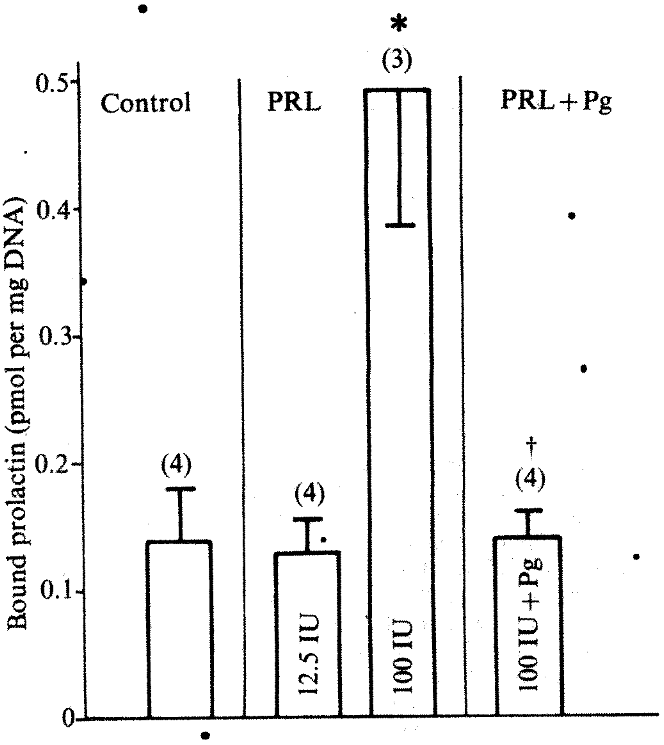

Fig. 2 Effect of prolactin (PRL) and prolactin+progesterone (PRL+Pg) injections on prolactin receptors concentrations in the mammary gland of 15 d pseudopregnant rabbits. Each point represents the means±s.e. for the number of animals indicated in parentheses. The affinity constant ($K_a = 3 \times 10^9\ \mathrm{M}^{-1}$) did not vary. *Significantly higher than control ($P < 0.025$). †Significantly lower than 100 IU prolactin treated animals ($P < 0.025$) and not different from control value.

progesterone on lactogenesis[2–4] to an additional parameter–receptor concentration; it is not known whether this parameter is causally related to the various amplification mechanisms which ultimately cooperate in lactogenesis, by modulating the information available to the target cell, or whether it is just part of the pleotypic action of prolactin on this cells protein synthesis.

It is thought that prolactin and progesterone receptors are both triggered initially by oestrogens[7,13], and hence might first appear in the mammary gland simultaneously with the oestradiol peak at the onset of pseudopregnancy. It is known that oestradiol induces its own receptors[14] and that prolactin at least amplifies its own receptor levels in the liver[8] and mammary gland (present results). In addition, progesterone counteracts both the self-regulated increase of oestradiol receptors[15] and the increase of prolactin receptors in the mammary gland (present result). The interrelations between the circulating concentrations of these hormones and the levels of their receptors thus seem complex and it may be relevant to emphasise that the prolactin effect on the pseudopregnant rabbit (Fig. 2) might be increased if this hormone were injected at a period when luteolysis should already have begun, resulting in lower progesterone levels. This view is supported by the obvious requirement for a specific hormonal environment (high prolactin levels, a cortisol peak and decreasing progesterone levels) for the efficient induction of prolactin receptors at parturition (Fig. 1).

The mechanisms by which prolactin and progesterone regulate prolactin receptors are a matter of conjecture and it is doubtful that these hormones act at precisely the same level on the complex sequence of events which result in the synthesis, intracellular transport and possible removal of the receptors. The so-called down regulation (that is, lowering of receptor levels by its specific hormone) has been shown to be due to accelerated removal or breakdown of the hormone–receptor complex (possibly by endocytosis) at least in the case of insulin[16,17]. This scheme should not apply to prolactin, which has just the opposite effect on its receptors. It remains to be shown to what extent this increase of receptors is due to an unmasking or recombination of previously inactive or segregated receptor molecules or to a *de novo* synthesis of these molecules.

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Alteration in glucocorticoid binding site number during the cell cycle in HeLa cells

GLUCOCORTICOID stimulation of alkaline phosphatase (AP) activity in HeLa cells is believed to be mediated by steroid receptors[1,2]. When synchronised HeLa cells are exposed to glucocorticoids, an increase in AP activity is initiated during the synthesis phase of the cell cycle[3]. This 'S-phase dependency' suggests that the necessary components for cellular response to glucocorticoids are produced or altered during this time. Because the interaction of the steroid with its cytoplasmic receptor seems to be the first major intracellular event of the action of glucocorticoids, we have measured the glucocorticoid receptors present throughout the cell cycle in synchronised HeLa cells. We report here that although receptors are present throughout the cycle, the number per cell varies markedly within the cell cycle in both cytosols and whole cells.

HeLa S3 cells (adapted to suspension culture by Phillip Marcus, Albert Einstein Medical School) were maintained at a density between 2×10^5 and 6×10^5 cells per ml. The cells were synchronised by the double thymidine block procedure[4,5]. Samples of cells for receptor analysis were collected from the synchronised cultures at late G_1, S, mitosis and early G_1. Scatchard analysis of glucocortcoid-binding in HeLa cell cytosols was performed using dextran-coated charcoal to remove free steroid. Uptake by whole cells was studied using a modification of the techniques described by Munck and Wira[6].

As Fig. 1 shows, glucocorticoid receptors were present in cytoplasmic fractions at all stages of the cell cycle studied. Scatchard analyses of saturation curves indicate a single class of high affinity binding sites (Fig. 1). The affinities of the cytoplasmic receptors for dexamethasone did not vary by more than a factor of two and they agree with published results for dexamethasone-binding in other tissues[11]. The number of cytoplasmic dexamethasone-binding sites per cell doubled between late G_1 and S (Fig. 1). Cells collected at S showed maximal rates of incorporation of ^{3}H-thymidine. Between mid-S-phase and mitosis (4 h) there was a considerable reduction in cytoplasmic dexamethasone-binding per cell, which correlated with a doubling of cell number. By early G_1 there had been a small increase in binding. We have observed this pattern of alteration in numbers of glucocorticoid receptors in seven identical synchrony experiments. The data suggest that the number of cytoplasmic glucocorticoid receptors per cell doubles before mitosis, with less than half the complement available for assay in the daughter cells.

When the number of glucocorticoid-binding sites is expressed as a function of other biochemical parameters (DNA, protein and cell volume) (Table 1), a similar, cell cycle-dependent variation is seen, although it is less pronounced. This variation was also observed in all seven experiments.

We have assessed dexamethasone-binding after incubation of whole cells with the steroid at both 3 and 37 °C. Figure 2 shows the results of one of four nuclear translocation experiments performed on cells from separate synchronised populations.

As in studies of ^{3}H-dexamethasone binding in cytosols, the level of saturable ^{3}H-dexamethasone-binding in whole cells showed cell cycle dependence. Figure 2*a* shows that, as in other systems[11] most glucocorticoid-binding was restricted to the cytoplasmic fractions after incubation at 3 °C, with very little binding associated with the nucleus. Cytoplasmic binding in whole cells at 3 °C significantly increased between late G_1 and synthesis and decreased to $\simeq 1/2$ synthesis values after mitosis. Nuclear binding at 3 °C did not show similar cell cycle dependence. When cells were warmed to 37 °C (Fig, 2*b*) there was a significant increase in nuclear binding at each stage of the cell cycle, with a concomitant reduction in the level of cytoplasmic binding. Both cytoplasmic and nuclear binding at 37 °C showed the cell cycle dependence observed with cytosol at 3 °C (Fig. 1) and whole cell cytoplasmic fractions at 3 °C (Fig. 2*a*). Total-cell binding (cytoplasmic and nuclear) was similar when measured at 3 °C (Fig. 2*a*) or at 37 °C after 2 h at 3 °C (Fig.

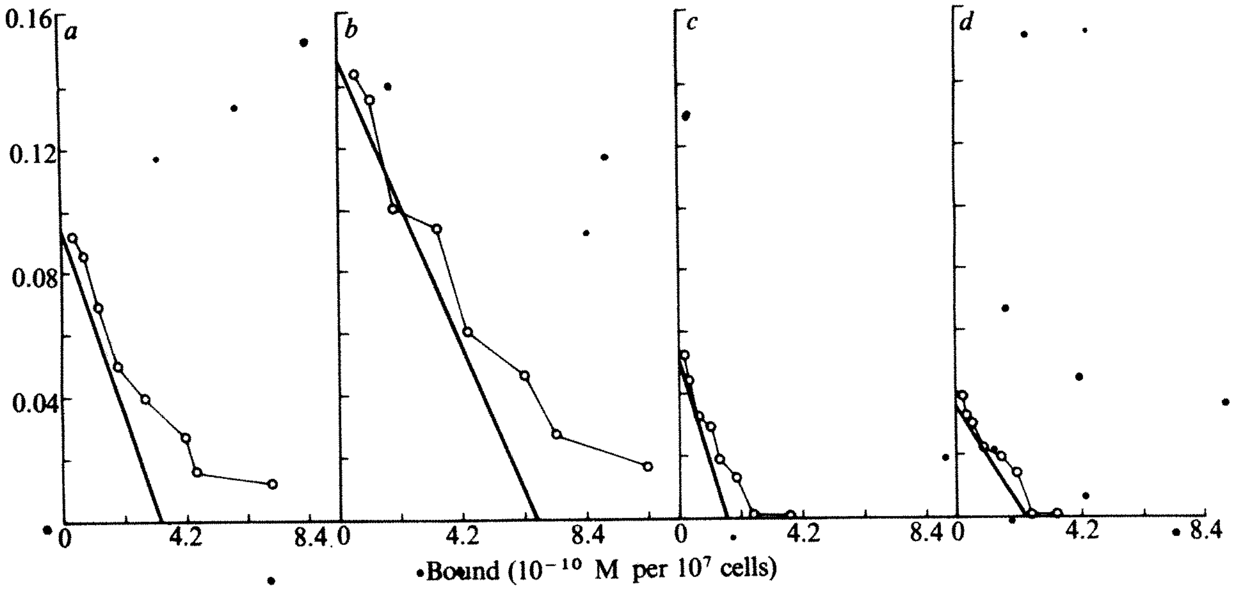

Fig. 1 Scatchard analysis of glucocorticoid—binding in HeLa cell cytosols. HeLa cells were grown in Joklik's modified Eagle's medium supplemented with 3.5% each of calf and foetal calf serum in the presence of kanamycin (10 μg ml^{-1}) in suspension culture at 37 °C. The cells have a doubling time of about 20 h. The cells were synchronised by double thymidine block[4,5]. They were grown in 2 mM thymidine for 16 h, followed by growth for 10 h in thymidine-free medium. Thymidine treatment was repeated for a further 14 h and the cells were washed free of thymidine. Growth was then resumed in thymidine-free medium; time of release from the second thymidine block is time 0. During late G_1 S, mitosis and early G_1, 200-ml samples of cells were collected and centrifuged at 600*g* for 5 min and the resulting pellet was resuspended in 1.5 ml of medium containing 10% glycerol. After a period of slow cooling, cells were kept frozen in liquid nitrogen. At the time of assay, cells were thawed at 23 °C and washed with 50 ml of serum-free medium. Freezing and thawing had no effect on receptor affinity and binding sites per cell when tested with randomly growing cells. The thawed HeLa cells were centrifuged and resuspended in 2.5 ml Joklik's medium (without serum) at room temperature and three 200-μl samples were removed for determination of cell numbers, DNA[7] and total cell protein[8]. The cells were then pelleted by centrifugation at 600*g*, the medium was aspirated off and 3.0 ml of ice-cold Tris-EDTA buffer (0.01 M Tris, 0.0015 M Na_2 EDTA *p*H 8.0) was added to each pellet. Cell pellets were homogenised at 0–3 °C with three 5-s bursts from a Polytron (Brinkman Instruments) at setting 5. All subsequent procedures were performed at 3 °C. The homogenates were centrifuged at 12,500*g* for 4 min in an Eppendorf model 5411 microfuge. The supernatants (cytosol) were decanted into glass tubes and divided into 50-μl samples of eight different concentrations of ^{3}H-dexamethasone (prepared in 0.9% NaCl) from 1×10^{-7} M to 1×10^{-10} M. The cytosols were incubated for 90 min at 3 °C' This time was sufficient to attain near-maximal dexamethasone-binding in cytosol from randomly growing HeLa cells, in mammary gland cytosol[9] and in muscle cytosol[10]. After this incubation, 100-μl samples of dextran-coated charcoal (1% Norit A and 0.1% dextran type 60 C in 1.5 mM $MgCl_2$) was added to each sample. The tubes were then vortexed and left standing to incubate for 15 min, and subsequently centrifuged at 12,500*g* for 2 min. Samples of 75 μl of the supernatant were placed into 5 ml of Bray's solution for determination of radioactivity. Duplicate 5-μl samples of the original ^{3}H-dexamethasone solutions used for incubation with cytosol were counted for determination of total steroid concentrations. The percentage of cells in mitosis was determined by monitoring a parallel culture of synchronised cells growing in the presence of colchicine (0.2 μg ml^{-1}). Cells from growing culture were counted microscopically in a haemocytometer. Radioactivity was determined in a Packard (3310) liquid scintillation spectrometer with a 25% efficiency for tritium. Each cytosol preparation was analysed for protein by the method of Lowry[9]. DNA was measured by a microfluorometric assay[10]. The cytocrit of each cell suspension was determined using a Drummond microhaematocrit, *a*, Late GI = 24,802 sites per cell; *b*, synthesis = 46,950 sites per cell; *c*, mitosis = 12,040 sites per cell; *d*, early GI = 16,615 sites per cell. The *K* dissociations ranged from 1.3×10^{-8} M to 2.6×10^{-8} M.

2*b*), indicating that equilibrium was probably attained within 2 h at 3 °C during each stage of the cell cycle.

Our results show that glucocorticoid-binding occurs in HeLa S3 cells throughout the cell cycle when measured in cytosols and in cytoplasmic and nuclear fractions of whole cells. The 'S-phase dependency' of stimulation of AP activity therefore cannot be attributed to an absence of glucocorticoid receptors or the failure of the steroid receptor complex to be translocated to the nucleus at other stages of the cell cycle. Although it is possible that a critical number of receptors is needed for AP stimulation, other factors are more probably involved, since some cells with fewer binding sites are responsive to glucocorticoid stimulation[11]. Of the factors that involve the receptor, the following are likely candidates: (1) modification of the receptor itself (not at the binding site); (2) lack of functional nuclear acceptor sites, or (3) an altered nuclear processing of the steroid receptor complex. These can all show cell cycle dependence.

We have demonstrated that in the conditions we used to synchronise HeLa cells, the number of cytoplasmic and nuclear dexamethasone-binding sites is regulated in a specific manner in relation to the cell cycle. The simplest interpretation of the observed doubling of the number of receptors at S is that this represents the synthesis of new receptor for passage to daughter cells. Histone synthesis at S is an example of a regulatory component that is synthesised at one phase of the cell cycle[12]. The increased number of receptors, however, may not result from the synthesis of new receptors but could reflect activation of a proreceptor, differential degradation of receptor molecules or

Table 1 Relation of cell cycle stage to cytoplasmic receptor concentration per unit of protein, DNA and cell volume

Cell cycle stage	Late G_1	S	Mitosis	Early G_1
Moles bound				
per 100 mg total protein	1.44×10^{-13}	2.0×10^{-13}	1.0×10^{-13}	1.70×10^{-13}
per 100 mg cytosol protein	1.45×10^{-13}	2.1×10^{-13}	1.5×10^{-13}	2.15×10^{-13}
per 100 μg DNA	1.42×10^{-13}	1.71×10^{-13}	0.68×10^{-13}	0.77×10^{-13}
per ml of packed cells	20×10^{-13}	24×10^{-13}	12×10^{-13}	22×10^{-13}

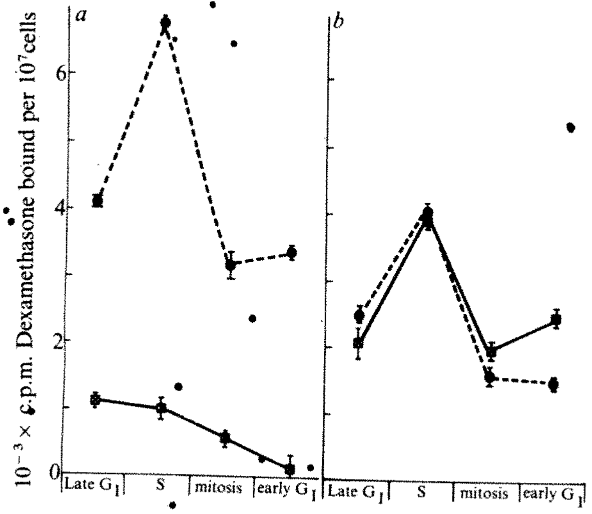

Fig. 2 Saturable cytoplasmic and nuclear ^3H-dexamethasone-binding in synchronised HeLa cells after incubation of whole cells with steroid. The thawed HeLa cells were suspended in 1.0 ml of ice-cold Joklik's medium without serum. Samples of 400 μl of cell suspensions from each stage of the cell cycle were incubated for 2 h at 3 °C (*a*) or 2 h at 3 °C plus 30 min at 37 °C (*b*) with $\simeq 1 \times 10^{-8}$ M ^3H-dexamethasone alone or ^3H-dexamethasone plus 1×10^{-6} M unlabelled dexamethasone. Saturable dexamethasone-binding was considered to be the difference between the observed binding with ^3H-dexamethasone alone and ^3H-dexamethasone plus unlabelled dexamethasone. Cytoplasmic (●) and nuclear (□) binding were measured in 20-μl samples of cells after cell lysis in dextran-coated charcoal (cytoplasmic) or 1.5 mM $MgCl_2$ (nuclear) as before[6]. A rapid freeze-thaw modification was used to ensure breakage of the cells. The values shown are the means ± s.e. of triplicate determinations of the saturable binding fraction from a single synchrony experiment.

masking or unmasking of the binding sites. If the doubling of receptor number between late G_1 and S represents synthesis of new receptors, these cells may provide an appropriate system for the isolation of receptor mRNA.

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Biological activities of prostaglandin analogues and metabolites on bone in organ culture

PROSTAGLANDINS, especially the E-series, are potent stimulators of bone resorption in several *in vitro* systems[1–4]. Studies with two experimental tumours ($HSDM_1$ mouse fibrosarcoma and VX_2 rabbit carcinoma) have indicated that excessive synthesis and secretion of prostaglandin E_2 (PGE_2) by the neoplastic cells is associated with and seems likely to be responsible for the hypercalcaemia observed in these tumour-bearing animals[2,5–8]. A similar hypercalcaemic syndrome occurs in some patients with cancer, is associated with excessive PGE_2 metabolite excretion in urine and is reversed at least in part by treatment with aspirin-like drugs[9,10]. Finally, intravenous infusion of PGE_2 in intact, unanaesthetised rats causes an increase of plasma calcium concentration[11]. The most straightforward interpretation of these findings is that PGE_2 is secreted by certain tumour cells into plasma in such quantity that the steady-state plasma concentration of PGE_2 is sufficiently high to stimulate osseous target cells to resorb bone. An alernative interpretation is that unmeasured intermediates in arachidonic acid metabolism or PGE_2 metabolites have sufficient bone resorption-stimulating activity to be the agent acting on bone cells. This hypothesis gains a degree of support from knowledge that some prostaglandin metabolites have longer half lives in plasma than PGE_2 itself[12]. We have now measured the biological activities of several endoperoxide analogues and PGE_2 metabolites in a bone culture assay system and compared their potencies with that of PGE_2.

The bone resorption-stimulating activities of three prostaglandin endoperoxide analogues are shown in Table 1. Azo-PG III, which stimulates platelet aggregation, release of 5-hydroxytryptamine and contraction of isolated rabbit aorta[13], did not stimulate bone resorption at doses up to 10 μg ml^{-1}. The other two analogues were only active;

Table 1 Effects of prostaglandin endoperoxide analogues U-44069 U-46619 and azo-PG III on bone resorption

Experiment	Treatment	Dose (μg ml^{-1})	Medium calcium (mg per 100 ml)
1	None	—	7.6±0.43
	PGE_2	0.005	8.6±0.43
	PGE_2	0.025	12.1±0.43*
	U-44069	4	8.1±0.43
	U-44069	20	11.4±0.43*
2	None	—	7.6±0.36
	PGE_2	0.004	9.1±0.36†
	PGE_2	0.050	13.8±0.36*
	U-44069	10	10.1±0.36*
	U-46619	10	8.6±0.36‡
3	None	—	7.0±0.43
	PGE_2	0.05	12.4±0.38*
	U-44069	15	11.0±0.38*
	U-46619	15	8.4±0.38‡
4	None	—	6.8±0.50
	PGE_2	0.01	8.8±0.50†
	PGE_2	0.10	10.3±0.50*
	Azo-PG III	5	6.8±0.50
	Azo-PG III	1	5.7±0.50
	Azo-PG III	0.50	6.1±0.50
	Azo-PG III	0.10	6.2±0.50
5	None	—	6.2±0.46
	PGE_2	0.01	9.1±0.46*
	PGE_2	0.10	10.6±0.46*
	Azo-PG III	10	6.4±0.46

Bone resorption was determined by measuring the release of calcium (^{40}Ca) from neonatal mouse calvaria in organ culture[2,14]. Medium total calcium concentration was measured with a Corning model 940 calcium analyser. Medium was Dulbecco's modified Eagle's medium supplemented either with bovine serum albumin (5 mg ml^{-1}) or heat-inactivated (60° C), 1 h horse serum (15% final concentration) and foetal calf serum (2.5% final concentration). Results were essentially the same in both kinds of medium. Treatment groups consisted of three or four bones each. Results given are mean values for medium total calcium ± s.e. at the end of 48 h of treatment with the compounds tested. U-44069 is (15S)-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid (lot no. 12344-JMB-85). U-46619 is (15S)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid (lot no. 12344-JMB-109). Azo-PG III is (5Z, 9α, 11α, 13E, 15S)-9,11-azo-15-hydroxyprosta-5,13-dienoic acid[13]. The data in each experiment were subjected to an analysis of variance and s.e. was calculated from the residual error form of that analysis.

*Statistical difference from control mean $P < 0.001$.
†Statistical difference from control mean $P < 0.01$.
‡Statistical difference from control mean $P < 0.05$.

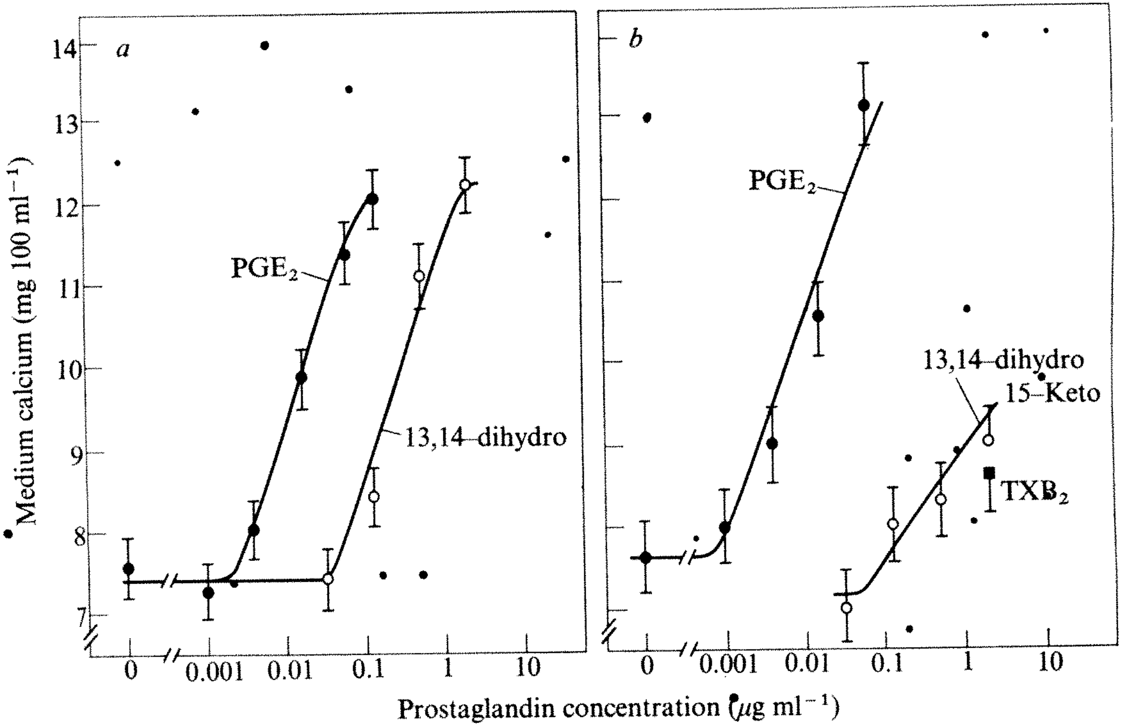

Fig. 1 Effects of increasing concentrations of PGE$_2$, 13,14-dihydro-PGE$_2$ and 13,14-dihydro-15-keto-PGE$_2$ on bone resorption in organ culture. Neonatal mouse calvaria were cultured and analysed as described in Table 1 except that both experiments were performed in heat-inactivated serum medium. Furthermore, the calvaria in these experiments were free floating instead of attached to cover slips as described in the original procedure[2, 14]. This modification simplifies the procedure without affecting the outcome. Each point gives the mean value for groups of three or four bones, and the vertical bars give the s.e. at the end of 48 h of treatment. The s.e. were calculated from an analysis of variance. *a*, PGE$_2$ (●); 13,14-dihydro-PGE$_2$ lot no. 12874-JHK-52A (○). *b*, PGE$_2$ (●); 13,14-dihydro-15-keto-PGE$_2$, lot no. 12277-JHK-122A (○); thromboxane B$_2$, lot no. 13066-RWJ-38A (■).

U-44069 and U-46619 were about 0.08% and 0.06%, respectively, as active PGE$_2$. None of these analogues was an effective antagonist of PGE$_2$-stimulated bone resorption (data not shown).

The bone absorption-stimulating activities of the PGE$_2$ metabolites 13,14-dihydro-PGE$_2$ and 13,14-dihydro-15-keto-PGE$_2$ were about 6.6% and 0.20%, respectively, as active as PGE$_2$ (Fig. 1). Thromboxane B$_2$ had even less biological activity than 13,14-dihydro-15-keto-PGE$_2$.

Our results indicate that the bone resorption-stimulating activity of PGE$_2$ is at least 1,000 times greater than that of the three prostaglandin endoperoxide analogues tested. PGE$_2$ is also about 15 times more potent than 13,14-dihydro-PGE$_2$ and about 500 times more potent than 13,14-dihydro-15-keto-PGE$_2$, although an accurate estimate of the potency of the latter metabolite was not possible because its dose–response curve was not parallel to that of PGE$_2$ (Fig. 1).

Because of their relatively low biological activities, if any of the metabolites were responsible for the bone resorption and hypercalcaemia seen in HSDM$_1$ and VX$_2$ tumour-bearing animals, the half lives of these compounds in plasma would need to be considerably greater than that of PGE$_2$, thus making it possible for them to accumulate to very high levels in plasma and extracellular fluid. The half life of PGE$_2$ is very short, probably less than 1 min[12]. The half life of native prostaglandin endoperoxides in aqueous media is about 5 min[12] and probably less in plasma although this has not been determined accurately. The half life in plasma of 13,14-dihydro-15-keto-PGE$_2$ is about 8 min and that of 13,14-dihydro-PGE$_2$ is not known[12]. Of the compounds tested, the most likely mediator of bone resorption, other than PGE$_2$, would be 13,14-dihydro-PGE$_2$ because its biological activity was the highest, about 6–7% of PGE$_2$. Unfortunately, no data are available on plasma concentrations of 13,14-dihydro-PGE$_2$ in control or tumour-bearing animals because there are no assays for this metabolite. We have reported that in tumour-bearing mice, plasma concentrations of 13,14-dihydro-15-keto-PGE$_2$ are considerably higher than corresponding levels of PGE$_2$[8]. This increment was not sufficiently large to cause hypercalcaemia in the mice because of the very low bone resorption-stimulating activity of 13,14-dihydro-15-keto-PGE$_2$. Seyberth *et al.* have also noted a greater increase in plasma 13,14-dihydro-15-keto-PGE$_2$ than PGE$_2$ in rabbits bearing the VX$_2$ carcinoma[15], and L. G. Raisz has observed (personal communication), using foetal rat long bones in culture, that 13,14-dihydro-PGE$_2$ and 13,14-dihydro-15-keto-PGE$_2$ are substantially less active stimulators of bone resorption than PGE$_2$.

Although our data do not rule out the possibility that prostaglandin endoperoxides or metabolites are the mediators of bone resorption and hypercalcaemia in the HSDM$_1$ and VX$_2$ model systems, we believe that the data available strongly suggest that PGE$_2$ is the mediator. Measurements of 13,14-dihydro-PGE$_2$ in plasma will provide more evidence when they are available.

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Inhibition of masculine differentiation in male offspring of rabbits actively immunised against testosterone before pregnancy

ACTIVE immunisation of male rabbits with testosterone–protein conjugates results in the production of antibodies capable of neutralising the biologic activity of endogenous testosterone[1]. Due to the reduction of free, biologically active testosterone caused by almost total binding to antibodies the entry of this hormone into the target cells is inhibited, the metabolic clearance rate is decreased, the negative feedback to the hypothalamus is interrupted and pituitary gonadotropin release is increased. Thus, in spite of enormously elevated total plasma testosterone concentrations, atrophy of the prostate, paraprostate and seminal vesicles can be observed in suitably immunised male rabbits. In female rats actively immunised against testosterone Hillier *et al.*[2] also observed—despite significantly elevated total plasma testosterone concentrations —a considerable reduction of the free testosterone fraction and an elevation of FSH. Histological examination of these animals revealed development of polycystic ovaries, similar to those seen in the Stein–Leventhal syndrome in humans. These reports prompted us to study whether it is possible to fertilise female rabbits after active immunisation against testosterone, and whether in pregnancy the antibodies pass through the placenta in sufficient concentrations to neutralise the biologic activity of testosterone secreted by the foetal testes, thus selectively inhibiting the testosterone-dependent steps of male sex differentiation. We found inhibition of masculine differentiation in offspring of rabbits actively immunised against testosterone before pregnancy.

We immunised eight mature female rabbits by repeated subcutaneous injections of 2 mg testosterone-3-bovine serum albumin (T-3-BSA) dissolved in 1 ml of 0.9% saline at intervals of 2–6 weeks. For the first three injections, the antigen solution was emulsified in 1 ml of Freund's complete adjuvant. A control group of four animals was immunised against unconjugated bovine serum albumin (BSA) in an identical schedule. All animals injected with T-3-BSA developed highly specific antibodies against testosterone. Three months after beginning immunisation the titres ranged from 1 : 4,000 to 1 : 40,000 and increased after further booster injections. No antibodies to testosterone could be detected in the controls and their plasma testosterone concentrations (7–26 ng per 100 ml) were unaffected by the immunisation with BSA. In testosterone-immunised animals, however, the increase in antibody concentration was paralleled by a striking elevation of total plasma testosterone from an average of 12 ng per 100 ml before immunisation to 630–1,450 ng per 100 ml when anibody titres above 1 : 40,000 were reached. Regardless of these high testosterone levels, which exceed the normal range of male rabbits[1], we tried to mate these animals. After several fruitless attempts mating was successful in five of them. Altogether they gave birth to 19 males and 16 females. Throughout pregnancy the antibody titres varied from 1 : 15,000 to 1 : 40,000; the testosterone binding capacity at 0° C ranged from 130 to 220 μg per 100 ml. The four control animals produced 11 males and 16 females. Immediately after delivery all newborns were bled and blood was collected separately. The genital tract was fixed in Bouin's mixture, embedded in paraffin, cut transversely and stained with haematoxylin eosin.

In all neonates from animals immunised with testosterone high concentrations of antibodies against testosterone were found, with specificity and association constants identical to those in the maternal plasma. The testosterone binding capacity was about 40% of that of the corresponding mother. Plasma testosterone levels were very high with mean values of 1,600 ng per 100 ml in the females and 24,000 ng per 100 ml in males. The mean testosterone levels in the female and male control neonates, in contrast, were 15 and 18 ng per 100 ml, respectively, which resembled those of normal neonatal rabbits.

In spite of their enormously elevated testosterone levels, the experimental female neonates showed normal female sex differentiation; the males, however, revealed genital malformations which can be traced back to the inactivation of testosterone by antibody binding, resulting in feminisation of internal and external genitalia. The most striking finding was the underdeveloped accessory sexual glands. In most animals the prostate and paraprostate were totally absent and the vesicular glands were reduced to rudimentary buds (Fig. 1). The bulbo-urethral glands, which in males are normally more extensively developed than in females, showed the extent of female glands only. Seminal vesicles were present in all experimental males; however, in most of them they were less developed than in normal neonates. The Wolffian ducts were severely affected and were at the point of regression (Fig. 2). The epithelium was irregular and flat, the basal membrane had disappeared and the surrounding mesenchyme penetrated into the epithelium. Pycnosis, chromatolysis and other signs of imminent cell death predominated. The phallus of most males immunologically deprived of testosterone was also feminine (Fig. 3). The urethra corresponded to the female sinus urogenitalis rather than to the urethra of normal males. The corpus fibrosum was reduced to the

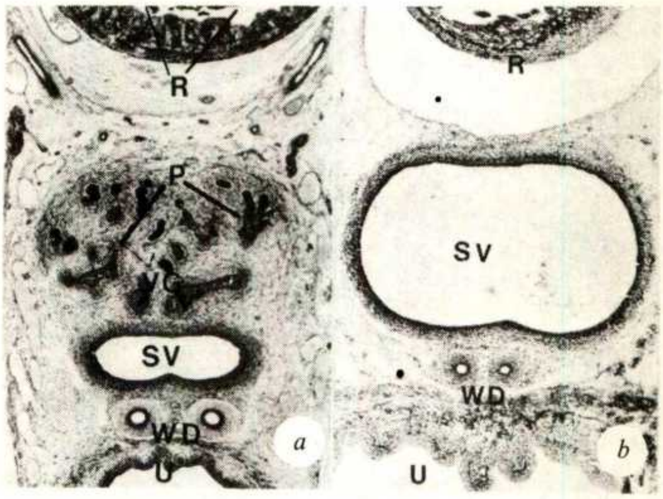

Fig. 1 Cross sections of male newborn rabbits at the level of prostate and vesicular gland. *a*, Male control (showing no difference from normal males); *b*, male immunologically deprived of testosterone. R, rectum; U, urethra; P, prostate; VG, vesicular gland; SV, seminal vesicle; WD, Wolffian duct.

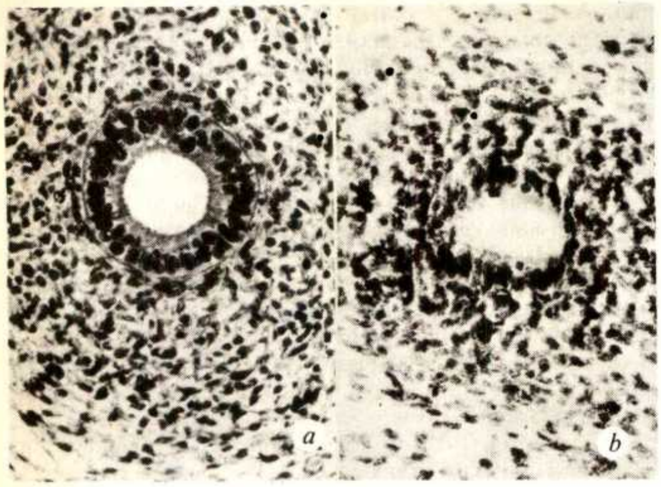

Fig. 2 Cross sections through Wolffian ducts of male newborn rabbits. *a*, Male control (showing no difference from normal males); *b*, male immunologically deprived of testosterone.

normal extent for females. As in females, the lamina glandaris was open ventrally, the urethra was located eccentrically, protruding into the perineum, and severe hypospadias could be observed. Despite these distinct manifestations of testosterone deficiency we never observed alternative development of female gonoducts. As in normal males the Müllerian ducts were completely inhibited. No abnormalities were detected in the testes, so withdrawal of testosterone as from the beginning of foetal development did not influence the gonadal differentiation. Microscopic examination of the control litters revealed normal sex differentiation in both sexes. The histological pictures were indistinguishable from those of normal female and male neonatal rabbits, respectively.

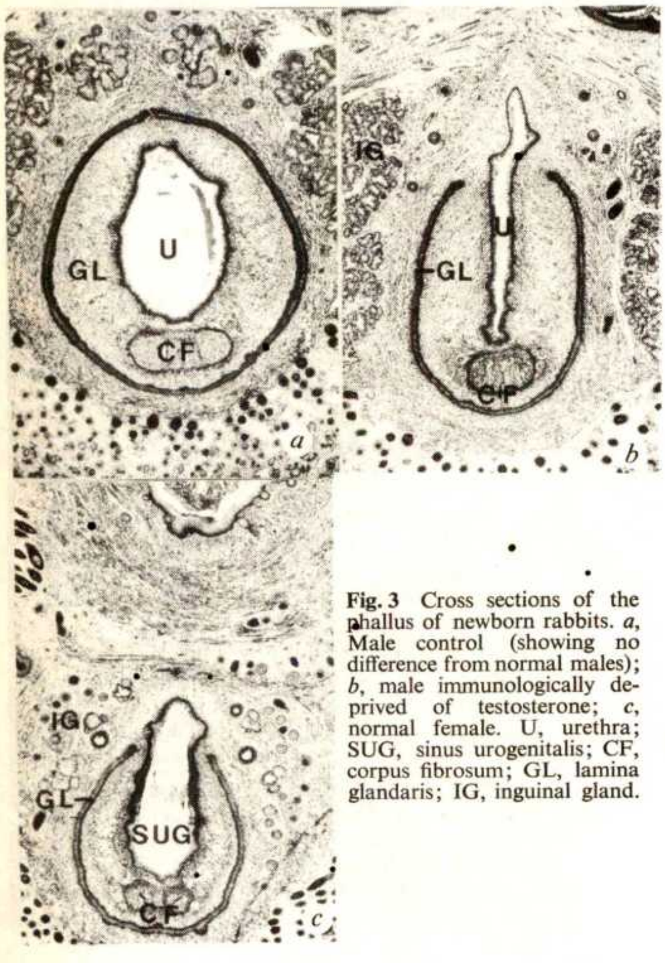

Fig. 3 Cross sections of the phallus of newborn rabbits. *a*, Male control (showing no difference from normal males); *b*, male immunologically deprived of testosterone; *c*, normal female. U, urethra; SUG, sinus urogenitalis; CF, corpus fibrosum; GL, lamina glandaris; IG, inguinal gland.

Our experiments indicate that hormones, which are essential for normal foetal development, can be neutralised in the foetuses by their respective antibodies raised in the mother, provided that the immunisation does not interfere with fertilisation. In rabbits active immunisation against testosterone does not preclude pregnancy. After passage of the placenta, the antibodies inhibit the entry of testosterone produced by the foetal testes into the cells of the developing target organs. In contrast to the experiments of Jost[3], who deprived male foetuses totally of testicular activity by castration *in utero*, our experiments resulted in the selective suppression of the androgenic effects of the foetal testes, leaving that testicular factor intact which controls Müllerian duct inhibition. Thus, with respect to the foetal genital tract the effects of maternal immunisation against testosterone are comparable to those of blocking the foetal androgens by treating the pregnant mothers with cyproterone acetate, as described by Neumann *et al.*[4] The 1,000-fold elevation of plasma testosterone in the male offspring of testosterone-immunised female rabbits suggests, however, that the antibodies exhibit additional important effects on foetal testosterone metabolism and regulation.

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Vitamin A deprivation and *Drosophila* photopigments

VITAMIN A deprivation causes a profound reduction in visual sensitivity and photopigment concentration in the eyes of both vertebrates and invertebrates[1,2]. In vertebrates vitamin A deprivation results in degeneration of photoreceptor cells[3] and decreases the number of intramembrane particles visualised in the disks of rod outer segments by freeze-fracture electron microscopy[4]. This and other evidence[5] suggests that these particles represent rhodopsin molecules. Invertebrate photoreceptors have similar, 8-nm intramembrane particles in the stacked microvilli which form their light-sensitive rhabdomeres[6,7]. The number of particles is comparable to the number of pigment molecules present[8] and is dependent on the state of light adaptation[9]. We describe here a study of the effects of vitamin A deprivation on the fine structure, photopigment content, and sensitivity of *Drosophila* photoreceptors. The results support the hypothesis that most of the intramembrane particles in invertebrate rhabdomeres, like those in vertebrate photoreceptors, are rhodopsin molecules.

D. melanogaster (Canton-Special strain) were deprived of vitamin A by raising sterilised eggs on Sang's synthetic medium C (ref. 10); control flies were reared identically except that β-carotene 1.25 mg ml^{-1} was added to the medium. White-eyed mutants (*w*) were employed in some experiments to eliminate screening pigments which interfere with spectrophotometric measurements. Fly heads were bisected, fixed for 2 h at 21 °C in half-strength fixative[11], and washed in cacodylate buffer. Preparations for conventional electron microscopy were postfixed in osmium tetroxide, embedded in epoxy plastic, sectioned, and stained with

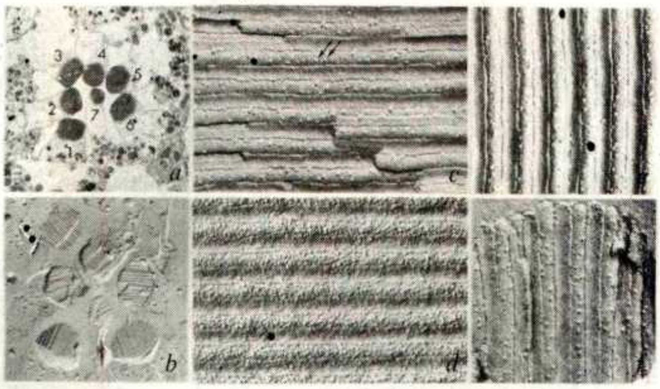

Fig. 1 Electron micrographs of *Drosophila* photoreceptors: *a*, tangential section through the central portion of an ommatidium in a vitamin A-deprived fly, showing cells R1–R6 and R7. The size and arrangement of the retinular cells and their rhabdomeres are normal (×2,400); *b*, freeze-fracture view similar to the above, demonstrating fracture faces in a vitamin A-deprived fly and the ability of the technique to demonstrate specific retinular cells (×4,000); *c*, high-magnification freeze-fracture view of rhabdomeric membranes of a peripheral photoreceptor in a vitamin A-deprived specimen—8-nm intramembrane particles (arrows) are very few in number (×43,000); *d*, freeze-fracture view of control rhabdomeric membranes, which are heavily studded with particles (×43,000); *e*, high-magnification photomicrograph of a cell R7 rhabdomere from a deprived animal, demonstrating a paucity of particles (×43,000); *f*, membrane fracture faces of a cell R8 from a deprived fly, showing a reduced intramembrane particle density (×43,000).

lead citrate. Freeze-fracture specimens were equilibrated for 8 h with 25% glycerol in *Drosophila* saline, freeze-fractured and etched for 1 min in a Balzers apparatus, and replicated by platinum and carbon shadowing. Specimens were examined with a Philips EM 301 and measurements made on prints at a magnification of 100,000×. Visual pigment content in homogenates prepared from exactly 100 heads each was assayed for light-induced absorbance changes in a dual-wavelength spectrophotometer[12]. Visual sensitivity was measured by determining the intensity of 470-nm light needed to elicit a criterion electroretinogram (ERG) response[12] of 5 mV.

Vitamin A-deprived *Drosophila* retinas appear normal in low-power electron micrographs (Fig. 1*a*, *b*) and rhabdomeric dimensions fall within the normal range. The number of 8-nm intramembrane particles in rhabdomeric microvilli, however, is decreased about fourfold in comparison with controls (Fig. 1*c*, *d*, Table 1). Spectrophotometric measurements reveal a greater than 70-fold reduction in photopigment level (Fig. 2, Table 1). Correspondingly, visual sensitivity as measured by the ERG is reduced approximately 80-fold by vitamin A deprivation (Table 1).

The normal size and shape of the rhabdomeric microvilli in vitamin A-deprived *Drosophila* show that a full complement of rhodopsin is not required for structural integrity of the photoreceptor membrane, a finding similar to that in *Aedes*[13] but contrasting with that in vertebrate rods[3] and moth photoreceptors[14]. The nature of the intramembrane particles remaining after vitamin A deprivation is unknown; because the reduction in rhodopsin measured spectrophotometrically or physiologically is much larger than the reduction in particle density, it is unlikely that the persisting particles represent rhodopsin molecules. The discrepancy of results obtained by the three techniques could be explained if some of the particles are not photopigments or if they are opsin molecules lacking chromophores. Nevertheless, the evidence indicates that the large majority of the 8-nm particles in the rhabdomeric membranes of normally reared flies are rhodopsin molecules.

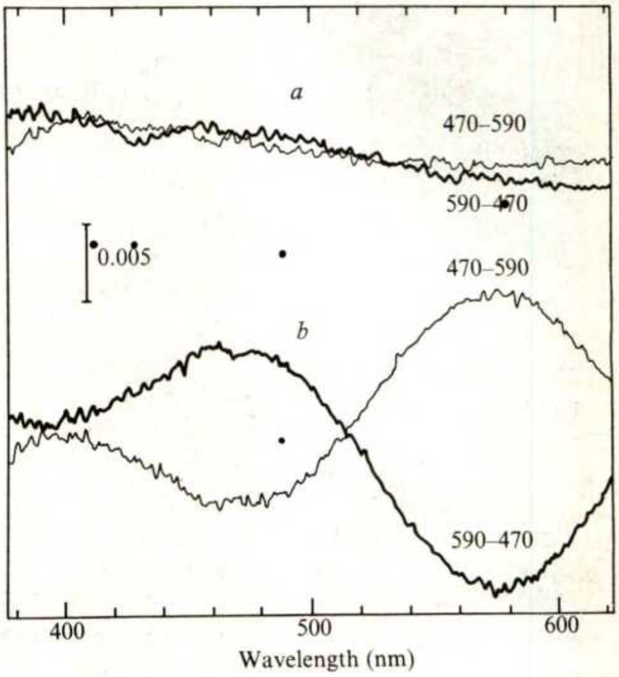

Fig. 2 Absorption spectra changes for *a*, vitamin A-deprived flies and *b*, control flies. The ordinate is the difference between the absorbances after 10 s actinic irradiations presented successively at 470 nm (2.7 mW cm^{-2}) and 590 nm (2.3 mW cm^{-2}; thin lines) or in the opposite order (thick lines).

Each subunit or ommatidium of the *Drosophila* compound eye contains eight photoreceptors which constitute three distinct classes, each with a different photopigment[12]. These three types correspond morphologically to the peripheral photoreceptors, cells R1–R6, which are blue receptors; the upper-central cell, R7, which is an ultraviolet receptor; and the lower-central cell, R8, which is a blue receptor with a different pigment from that in cells R1–R6. The preponderance of R1–R6 cell types in the retina

Table 1 Effect of vitamin A deprivation on the density of intramembrane particles, light-induced absorbance change, and electroretinogram sensitivity in *Drosophila*

	Vitamin A-deprived	Control	Deprived Control
Intramembrane particle density (particles per μm^2)*	1030 ± 31 ($n = 27$)	4214 ± 163 ($n = 6$)	0.24 ± 0.01
Light-induced absorbance change (ΔA)	< 0.0002 ($n = 2$)	0.015 ± 0.002 ($n = 2$)	< 0.01
Electroretinogram sensitivity (mV μW^{-1} cm^{-2})*	0.42 ± 0.07 ($n = 5$)	32 ± 5 ($n = 5$)	0.013 ± 0.003

Intramembrane particle densities were determined from counts of the 8-nm particles on rhabdomeric 'P' fracture faces and from areal measurements made with a Wang 600 planimetric computer. No corrections were made for the curvature of microvillar surfaces. Data from white-eyed and normally pigmented flies were not significantly different and were combined. Light-induced absorbance changes were measured with the sensitive dual-wavelength mode of the spectrophotometer at 470 and 580 nm; 10 s actinic irradiations were provided alternately at 470 nm (2.7 mW cm^{-2}) and 590 nm (2.3 mW cm^{-2}). Visual sensitivity was measured for a criterion electroretinogram response of 5 mV to 470 nm light. Data are given as means with standard errors of means; n is respectively the number of rhabdomeres scored, of pigment preparations scanned, and of flies studied.

*Difference significant at $P < 0.001$.

usually masks the contribution of R7 and R8 cells to spectrophotometric and ERG measurements, and thus prevents the determination of the chromophore in these cells. The reduction of intramembrane particles by vitamin A deprivation in all three receptor classes (Fig. 1*e*, *f*, Table 1) suggests, however, that the three spectrally distinct photopigments all use retinal (or its derivatives) as a chromophore. This result is consistent with other evidence[15] that the ultraviolet-sensitive photopigments of flies are retinal-based.

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Storage of cannabinoids by *Arctia caja* and *Zonocerus elegans* fed on chemically distinct strains of *Cannabis sativa*

THE larvae of the warningly coloured tiger moth *Arctia caja* (L.) (Lepidoptera) and the grasshopper *Zonocerus elegans* (Thunberg) (Acridoidea) are polyphagous feeders with a predilection for poisonous food plants. These species can sequester and store various secondary plant substances such as cardiac glycosides and pyrrolizidine alkaloids[1,2], which presumably function as predator deterrents. We report here that cannabinoids ingested with the leaves of *Cannabis sativa* L. are also stored by both insects.

There is good evidence that *C. sativa* contains at least two chemical races or strains, one rich in the psychoactive substance Δ-1-tetrahydrocannabinol (THC) and the other in the inactive cannabidiol (CBD)[3], and accordingly one of each type was used in our work.

A. caja larvae, derived from a single egg batch from a wild female caught at Ashton Wold, were allowed to feed freely either on a Mexican THC-rich strain (referred to as THC strain) or a Turkish CBD-rich strain (referred to as CBD strain), with no alternative food offered. Leaves from matched shoots of both strains were taken for analysis as controls. Feeding continued for 44 d at 24 °C and 50% relative humidity. Another batch of larvae was reared to the third instar on the CBD strain, then transferred to the THC strain. Controls were fed on *Senecio jacobaea* L. Only nine wild caught *Zonocerus elegans* nymphs (from Nigeria, where they were feeding on cassava) were available. Because we found the Mexican strain of *Cannabis* had proved lethal for hoppers of *Schistocerca gregaria* (Forsk.) we allowed *Z. elegans* to feed on both strains indiscriminately. They showed no preference but fed sparingly. One specimen died and the rest were killed after 6 week continuous feeding on *Cannabis*. For the last 3 d before analysis the diet was changed to grass to empty the gut of cannabinoids. Eight whole insects, four exuviae and a sample of frass were analysed.

The *A. caja* larvae fed on THC strain were stunted (Fig. 1) and did not survive beyond the third instar. Those fed on the CBD strain developed more slowly than the controls, but pupated successfully after 7–8 weeks of larval life.

About 30% of larvae reared to the third instar on CBD strain and then transferred to the THC strain developed and pupated successfully. Subsequently, further attempts were made to rear larvae on the THC strain, and on one occasion four specimens reached the fifth instar after 9 months' continuous feeding, and, of these, one pupated and the female which emerged mated and laid fertile eggs.

The vegetative leaves of the Mexican strain contained 19.3 mg of THC per g dry weight, while those of the Turkish strain contained 10.2 mg of CBD per g dry weight. Thus the greater toxicity of the Mexican strain may have been due to greater toxicity of THC or simply to a greater absolute amount of cannabinoid: other differences between the two types may contribute to the more lethal effect of the Mexican THC strain.

The larvae (starved for 24 h before analysis), their frass, exuviae and pupae were examined for cannabinoids. Insect samples were extracted for 18 h with ethanol at room temperature and the extracts were assayed as described before[4]. Quantitative identification of cannabinoids was checked by thin-layer chromatography[5]. The residue was heated under reflux with 0.1 N NCl for 1 h and then

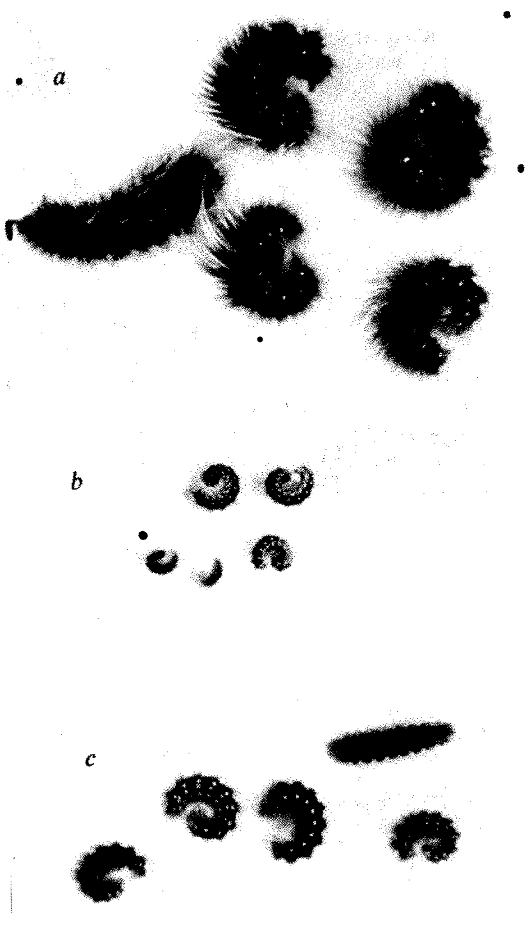

Fig. 1 Larvae reared for *a*, 32 d on ragwort (*Senecio jacobaea*); *b*, 32 d on the THC-rich strain of *Cannabis*; and *c*, 32 d on the CBD-rich strain of *Cannabis*.

Table 1 Cannabinoid contents (mg per g fresh weight) of *Arctia caja* fed on *Cannabis sativa**

	Feeding regime		
	CBD type throughout larval growth	CBD type to 3rd instar, and then THC type	THC type to death (3rd instar)
Larvae			
CBD	trace	0.21 (0.01)†	NE
THC	0	0.99 (0.07)	NE
Pupae			
CBD	0	0	NE
THC	0	0	NE
Exuviae	(mg per g dry weight)		
CBD	0.1 (0.0012)	0.28 (0.004)	0
THC	0	2.6 (0.04)	1.4 (0.003)
Frass	(mg per g dry weight)		
CBD	2.1	NE	0
THC	0	NE	3.0

*The coefficient of variation for a single assay involving all stages is only 2.7% (ref. 4). Where sufficient material was available it was analysed in duplicate.

†Figures in parentheses indicate the weight of cannabinoid in mg in each insect.

NE, not examined.

extracted with diethyl ether. This extract was also examined as above.

Table 1 shows that specimens reared throughout larval growth on CBD strain contained only traces of CBD, insufficient for quantitative analysis. Acid hydrolysis of the residue released a trace of a compound which gave a positive colour reaction for cannabinoids but did not correspond to any available standards on gas chromatography. Such traces were also found in the pupae and may represent a metabolite of CBD, present as a glucoside. CBD, as the acid, in which form it appears in the plant, was present in the exuviae (cast skins) at a concentration of 0.0012 mg per exuvia. On the other hand, larvae reared to the third instar on the CBD strain then transferred to the THC strain, stored more CBD per insect (0.01 mg) in the fifth instar than those fed solely on the CBD strain, and in addition each contained 0.07 mg of THC. This was also present mainly as the acid, but some decarboxylated THC was identified.

The exuviae of insects reared exclusively on the THC strain until death contained relatively high concentrations of THC acid. Thus, measured as mg of cannabinoid per g of insect material, the exuviae of insects reared on the THC strain contained more than ten times as much cannabinoid as those feeding on the CBD strain. Those reared to the third instar on the CBD strain and then switched to the THC strain contained 25 times as much.

In both sets of larvae at least 80% of the cannabinoids recovered was found in the relatively small sample of frass analysed (Table 1). This shows that they excrete much more cannabinoid than they store; however the concentration in the frass was much less than that in the plant material, which was the sole article of diet, so that most of the cannabinoid eaten must have been metabolised.

Most of the cannabinoid recovered from *Z. elegans* (Table 2) occurred in the frass. The weight of THC in the whole insect was remarkably similar to that in full grown *A. caja* larvae, but a much smaller proportion was stored in the integument. CBD was lacking in both the insects and their exuviae, although it occurred in the frass, thus indicating that *Z. elegans* can metabolise this compound efficiently.

The most striking aspect of the storage of THC by *A. caja* is the relatively large amounts concentrated in the cuticle. Thus the insect can rid itself of much of the toxic material not voided in the frass, but accumulates it between moults at a site where it could function as a 'predator deterrent'. Mice, which are voracious feeders on larval Lepidoptera, show a reduction of spontaneous activity when given an oral dose of 0.013–0.025 mg of pure THC (J. Pickens, unpublished). Thus, one larva containing 0.07 mg THC would be above the threshold dose for pharmacological activity. Although the accumulation of toxins in the cuticle has not been noted before, it may be a widespread method for the storage of deterrent secondary plant substances, especially in aposematic larvae which are followed by a cryptic adult stage[6].

When we offered newly hatched *A. caja* larvae (20 trials) a choice of leaves from both strains of the plant (matched for size, age and position on the stem) they showed a definite preference for the Mexican strain. This may be due to factors unconnected with the type and concentration of cannabinoids, but should these compounds exert a fatal fascination for tiger caterpillars it suggests another subtle system of insect control by plants.

Table 2 Cannabinoid contents of *Zonocerus elegans* fed on *C. sativa*

	THC content*	CBD content*
Whole insect (mg per insect)	0.06	0
Exuviae (mg per g dry weight)	0.006	0
Frass (mg per g dry weight)	2.95	0.97

*No additional cannabinoid was released by acid hydrolysis.

Our results demonstrate the versatility of moth and grasshopper in dealing with poisonous plant substances encountered during feeding. Both are preadapted to the storage of cannabinoids, for *C. sativa* has not been recorded as a food plant in nature. Because of widespread cultivation of *C. sativa* the ranges of the plant and *A. caja* now overlap. Furthermore, both store the more toxic THC rather than the relatively innocuous CBD. This is reminiscent of the aposematic butterfly *Danaus plexippus* (L.) and grasshopper *Poekilocerus bufonius* (Klug) which also store the more active cardiac glycosides[7] ingested with their food plants.

We thank Dr Elizabeth Bernays for the gift of living *Zonocerus*.

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Nature of recombinants produced by the RecBC and the RecF pathways in *Escherichia coli*

BASED on analysis of various recombination deficient mutants, at least three different pathways of chromosomal recombination have been suggested in *E. coli* K12: the RecBC, the RecF and the RecE pathways[1-3]. Our present knowledge of initial and final DNA structures and of the intervening events is very limited. Only some of the genes controlling each pathway have been identified[3,4]; the products of a few of these have been isolated and characterised[5-10]; little is known about the specific steps at which these products participate in the respective pathways. The role of exonuclease V, the product of the *recB* and *recC* genes, has been studied[11-15]. Recipients deficient in this enzyme give greatly reduced yields of viable recombinants[11], but support certain types of recombinational events with relatively high efficiency[12,13,15]. ExoV has thus been considered to be more important in determining viability of the recombinant cells than in the recombinational process itself. It has been suggested, on the basis of transformation and transduction experiments, that the recombinant products of the RecBC and the RecF pathways may be structurally different[16,17]. We present here results from conjugation crosses of *E. coli* K12 which demonstrate that the viable recombinants produced by the RecBC and the RecF pathways are significantly different in terms of the density of genetic exchanges present in them. A preliminary account of this work has already been presented[18].

A simple mathematical formulation of *E. coli* conjugation system[19] shows that the recombinational process in this system can be visualised as consisting of two operationally separable parts: (1) the initiation of the recombinational process at randomly distributed points on the chromosome, and (2) the development around these points of short regions of recombination (RRs), each of which may contain several genetic exchanges. Increase in the probability of initiation of the RRs is reflected in increased values of two-point recombination frequencies (R1) between pairs of markers, while increase in the mean number of exchanges per RR, is reflected in increased values of R2(1), which is a measure of the frequency of additional exchanges close to a selected exchange (Fig. 1). A comparison of R1 and R2(1) values in recombinants produced by the different recombinational pathways can thus give information about the differences present in the final products of these pathways and about the earlier events.

MD1763, a *pyrA* derivative of HfrH was mated with an *ara*, *thr*, *str-r*, F⁻ recipient, AB1157 and its *recB recC*, *recB recC sbcB*, and *recF* derivatives (Table 1). Selection was made for *ara*⁺ *str-r*, *ara*⁺ *pyrA*⁺ *str-r* and *ara*⁺ *pyrA*⁺ *thr*⁺ *str-r* recombinant types. The values of R1 and R2(1) calculated from these (Table 2) are strongly dependent on the recombinational pathways functioning in the recipients. It seems that the recombinational process controlled by the RecF pathway involves a higher density of exchange clusters along the chromosomal length and a higher number of recombinational exchanges per cluster as compared to the RecBC pathway.

The markers involved in the above intergenic cross are comparatively widely spaced, each of the two intervals (1 and 2) measuring a little more than half a minute on the standard genetic map of *E. coli* K12 (ref. 20). To see whether similar pathway-dependent effects are present in recombination involving closely spaced markers, two-point crosses involving markers, (1) within a single gene (*araC*), and (2) in two neighbouring genes (*araB* and *araC*) of a single operon, were performed for all the above-mentioned recipient backgrounds. R1 values were determined in each case (Table 3), and the effect of mutations in the RecBC or the RecF pathway on R1 values for closely spaced markers is essentially similar to that for widely spaced markers (Table 2).

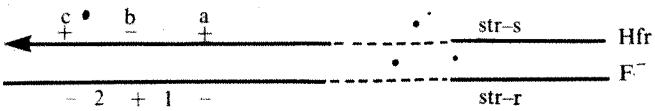

Fig. 1 Configuration of a typical three-point cross; R1 is the two-point recombination frequency in region 1 and is the number of $a^+b^+str\text{-}r$ recombinants per $a^+str\text{-}r$ recombinant. R2(1) is the frequency of recombination in region 2 in the subpopulation selected for recombination in region 1 and is given by the number of $a^+b^+c^+str\text{-}r$ recombinants per $a^+b^+str\text{-}r$ recombinant.

Table 2 Conjugational crosses between MD1763 donor strain and different recipient strains

Recipient strains	R1	R2(1)
AB1157	0.062±0.017	0.16±0.04
AB2470C	0.24 ±0.04	0.12±0.04
JC7623	0.24 ±0.05	0.25±0.05
JC9239	0.055±0.015	0.11±0.03

Conjugation experiments were performed as follows: exponentially growing cultures (2–4 × 10⁸ cells per ml) of donor and recipient cells were mixed in the ratio 1:5 and incubated for 5 min, then the mating mixtures were diluted 1:50 in liquid BT 8 nutrient broth containing tryptone) and allowed to mate for another 55 min with gentle shaking. The mating mixtures were then diluted 1:1 in BT containing 200 μg per ml⁻¹ streptomycin, blended and incubated with shaking for 4 h, then plated on prewarmed selection plates[14], spread with 0.7% topping agar and incubated for 40 h before counting the number of recombinant colonies. All operations were performed at 37°C. The results are averages of at least three experiments. The above cross is similar to the typical three-point cross shown in Fig. 1, with *ara-14*, *pyrA53* and *thr-1* representing markers *a*, *b* and *c*, respectively. Both R1 and R2(1) values are highest in JC7623, which is expected to produce most of its viable recombinants via the RecF pathway. The lowest values are obtained in JC9239, which lacks the RecF pathway and is mostly dependent on the RecBC pathway for recombinant production. The wild type AB1157 gives intermediate values for these frequencies, while AB2470C, which lacks exoV gives an R1 value comparable to JC7623 and an R2(1) value, somewhat lower than the wild type.

Table 1 *E. coli* K12 strains used in this study

Strains	Rec genotype *recB*	*recC*	*recF*	*sbcB*	Other markers	Sex	Source or ref.
AB1157	+	+	+	+	*thi-1*, *thr-1*, *leu-6*, *lacY1*, *mtl-1*, *xyl-5*, *ara-14*, *galK2*, *his-4*, *proA2*, *argE3*, *tsx-33*, *sup-37* (amber), *str-31*	F⁻	A. J. Clark
AB2470C	21	22	+	+	Same as AB1157	F⁻	J. Tomizawa
JC7623	21	22	+	15	Same as AB1157	F⁻	A. J. Clark
JC9239	+	+	143	+	Same as AB1157	F⁻	A. J. Clark
MD1763	+	+	+	+	*pyrA53*, *thi*	HfrH	19
MD2116	+	+	+	+	*araB113*, *trpR1*, *dra-2*	HfrH	This study
MD2121	+	+	+	+	*araC191*, *trpR1*, *dra-2*	HfrH	This study

MD2116 is an *araB* and MD2121 is an *araC* derivative of WP1767 (ref. 19); *araB* and *araC* mutations were isolated after mutagenesis with 2-aminopurine.

The comparable (three to fourfold) increase in R1 in AB2470C and JC7623 indicates that in the absence of exoV, the probability of initiating RR in a given chromosomal length increases. As most current models of recombination[21,22] postulate the presence of single-stranded breaks (nicks) in DNA as an essential prerequisite for initiating the recombinational process, it is reasonable to infer that, in the absence of exoV, the density of such nicks is higher. This is consistent with the suggestion[23] that *recB*$^+$ *recC*$^+$ gene products are involved in the repair of chain breaks that arise during cell growth. The alternative possibility that exoV has no effect on the mean density or distribution of RR and that the increase in R1 values is simply a reflection of higher selective survival (for some unknown reason) of the recombinant subpopulation with higher number of RR seems rather improbable: it would be difficult to understand why AB2470C and JC7623, which give widely different yields of viable recombinants, should give comparable increases in R1 values.

Table 3 Two-point recombination frequencies (R1) between pair of *ara* markers in different recipient strains

Recipient strains	Donor strains	
(*ara-14*)	MD2116 (*araB113*)	MD2121 (*araC191*)
AB1157	0.0095±0.0012	0.00047±0.00010
AB2470C	0.0253±0.0080	0.00138±0.00020
JC7623	0.0278±0.0060	0.00161±0.00003
JC9239	0.0090±0.0026	0.00031±0.00005

The experimental procedures were similar to those in Table 2. The order of *ara* mutations was *pyrA53 araB113 araC191 ara-14 leu*. To calculate the frequency of recombination between different *ara* markers, the nearby *leu* was used as a reference marker. The frequencies given represent ratios of *ara*$^+$ *str-r* recombinants to *leu*$^+$ *str-r* recombinants produced in the respective crosses and are averages of at least three independent experiments in each case.

The considerably higher value of R2(1) in JC7623 than in AB2470C indicates that the deficiency of exonuclease I increases the mean number of exchanges per RR which may be due either to an increased exchange density within these regions of recombination or to an increase in the mean length of these regions or both. The second alternative more easily explains the improved recovery of recombinants in the exoI deficient *recB recC* cells, and is consistent with results in P1-mediated transduction crosses[24]. These observations support a model (manuscript in preparation) which postulates that the RecBC pathway preferentially promotes integration of double-stranded donor DNA segments which may have short single-stranded ends, whereas the RecF pathway mostly promotes integration of single-stranded donor segments.

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High-LET radiations induce a large proportion of non-rejoining DNA breaks

THE mechanisms by which mammalian cells are killed by ionising radiation have not been explained at the molecular level and radiations with a high linear energy transfer (LET) can provide an important tool for investing these mechanisms. High-LET radiations, such as neutrons, π-mesons and low-energy heavy ions are known to kill bacteria[1,2], yeast[3,4], and mammalian cells *in vitro*[5–9] more efficiently per unit dose than radiations with diffuse patterns of ionization, or low-LETs, such as γ or X rays. Other radiobiological phenomena associated with high-LET radiations are a reduced effect of chemical modifiers, for example, oxygen[10], on cellular radiation sensitivity and reduction or loss of cellular ability to recover from radiation damage between split radiation doses[5,8]. Because of these and other attributes, including the favourable depth–dose distribution of heavy ions and π-mesons, high-LET radiations are being actively considered for use in cancer radiation therapy[11,12], and a thorough understanding of their biological effects is necessary for them to be used to advantage clinically. We report here that, over an LET range of 1–1953 keV μm^{-1}, there is an excellent correlation between the efficiency of exponential (single-hit) cell killing and the induction of non-rejoining DNA strand breaks, as measured on alkaline sucrose gradients. This correlation implies that non-rejoined breaks are a cause of cell death.

We measured DNA strand break induction and rejoining in Chinese hamster V79 S171 cells[13]. X rays (300 kVp, 1.6 mm Cu h.v.l.) and low-energy heavy ions from the Lawrence Berkeley Laboratory 88-inch cyclotron and heavy-ion linear accelerator provided radiations with the various LETs used (Table 1).

Table 1 Heavy ion parameters

Ion	Energy (MeV a.m.u.$^{-1}$)	LET* (keV μm^{-1})
Proton	4.0	9.4
Proton	1.15	27
^{9}Be	4.0	151
^{12}C	3.8	332
^{20}Ne	3.8	827
^{40}A†	3.9	1953
^{40}A	1.5	2900

*LET in water.
†Three experiments, with ion energies of 4.6, 4.2 and 3.0 MeV a.m.u.$^{-1}$ are grouped here.

As LET increased from 1 keV μm^{-1} with X rays to 1953 keV μm^{-1} with ^{40}A ions, DNA strand break induction decreased from $2.67 \pm 0.14 \times 10^{-4}$ to $0.48 \pm 0.03 \times 10^{-4}$ breaks per 10^8 daltons per rad (Fig. 1*a*). The percentage of these breaks that did not rejoin in post-irradiation incubation of $8\frac{1}{2}$ h or more, however, increased with LET from 1.4±0.4% for X rays to 17.9±1.5% at 1953 keV μm^{-1} (Fig. 1*b*).

The data of Fig. 1*a* and *b* were combined to obtain the efficiency for the induction of non-rejoining breaks, normalised to the value obtained for X rays. This yielded a relative efficiency for non-rejoining break induction as a function of LET (Fig. 2). The peaked response obtained is similar to that found for killing of some strains of bacteria[1,2] and yeast[3,4], for induction of mutations in yeast[14], for killing of mammalian cells *in vitro*[5–], and for induction of chromosome aberrations in mammalian cells *in vitro*[7].

Killing of mammalian cells by ionising radiation can be described in terms of two classes of radiation interactions[15–17]: an irreversible lethal class, which follows single-hit kinetics and leads to an initially non-zero, negative slope on log survival against dose curves; and a sub-lethal class, which may or may not be reversible ad which leads to a sigmoidal survival curve. Most of the high-LET, mammalian cell studies cited above demonstrate that the single-hit component of cell killing predominates for high-LET radiations, yielding linear survival curves on log plots.

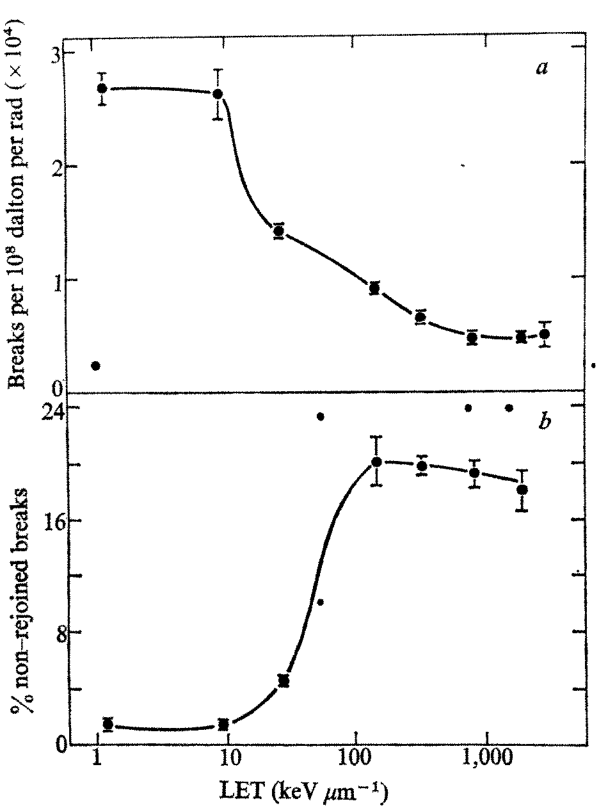

Fig. 1 Induction of DNA strand breaks by X ray and heavy ion irradiation. V79 S171 cells were grown as monolayers on glass dishes and labelled with ^{14}C-thymidine (0.07 μCi ml^{-1}, 57 Ci mol^{-1}) for 12–14 h. They were then exposed to X rays or to the heavy ion radiations indicated in Table 1, and allowed to grow for 0–12 h before collecting for gradient analysis. To eliminate the need for alkaline sucrose gradient analysis immediately, cells were frozen and gradient analysis was performed up to 1 month later. For collecting, cells were rinsed twice with citrate saline (0.75 M sodium citrate, 0.075 M sodium chloride) and 0.8 ml of citrate saline was added to cover the monolayer. The entire dish was then pressed against a methanol-coated thin aluminium plate resting on powdered dry ice. This gave very rapid freezing (within 2–3 s) of the cell monolayer and the citrate–saline solution that covered it. Dishes were placed in deep freeze (−76 °C) for storage. When gradient analysis was to be performed, cell dishes were thawed in 3–4 min at room temperature. Cells were detached from the surface by gentle repipetting of the citrate–saline solution covering them, and then an equal volume of lysis solution, consisting of 0.5 M NaOH, 0.02 M Na_2-EDTA and 0.1% NP-40 (non-ionic detergent) was added. Lysis was allowed to proceed for 1½–2 h, and 1.0 ml of the lysate was transferred to the top of an alkaline sucrose gradient. Transfer was performed with a wide-bore pipette tip connected by a Tygon tube to a motor-driven syringe. Alkaline sucrose gradients (5–20%) were formed from 5% sucrose containing 0.1 M NaOH, 0.9 M NaCl and 0.01 M Na_2-EDTA (*p*H 12.15) and 20% sucrose containing 0.15 M NaOH, 0.85 M NaCl and 0.1 M Na_2-EDTA (*p*H 11.20). Thirty min after the cell lysate was transferred, gradients were centrifuged at 19,700 r.p.m. in an SW 27 rotor for times that brought the peak of the DNA profiles approximately 75% of the distance down the tube. Thirty fractions per gradient were collected by puncturing the tube bottom, the radioactivity per fraction was counted, and molecular weights (weight average and number average) and the numbers of DNA breaks were calculated as before[27,28], using phage T4 DNA as a molecular weight standard. A number average molecular weight of approximately 2×10^8 was calculated for DNA from unirradiated V79 cells. *a*, Efficiency of induction of strand breaks. Cells were irradiated at 4 °C with doses between 3 and 45 krad and were analysed for strand breaks without subsequent incubation at 37 °C. *b*, Percentage of non-rejoining breaks, that is, DNA breaks in (*a*) that did not rejoin in 8½ h or more of post-irradiation incubation at 37 °C. These percentages were independent of dose (data not shown). Rejoining data were not obtained for the 2,900 keV μm^{-1} ^{40}A ion irradiations.

Todd[18,19] analysed extensive high-LET survival data for T-1 human kidney cells in terms of such single-hit and sigmoidal components. Relative efficiencies for single-hit killing, equivalent to relative efficiencies measured at high survival levels, can be calculated from Todd's data. These are in excellent agreement with the relative efficiencies for non-rejoining DNA break induction found here (Fig. 2). These non-rejoining break data are also in close agreement with other high-LET data for single-hit killing of Chang human liver cells[19] and Chinese hamster CH2B2 cells[7] (not shown). The implication is that non-rejoining DNA breaks cause single-hit, irreversible cell death. If this is true, then non-rejoining breaks would constitute the predominant lethal lesion for high-LET radiations. At low-LETs, their contribution to cell killing would depend upon such factors as dose and dose rate, becoming relatively more important, compared to sublethal damage, as dose or dose rate was reduced.

There is evidence to support the contention that non-rejoining DNA breaks might result in cell death. Some *Escherichia coli* mutants that cannot rejoin X-ray-induced DNA breaks exhibit extreme X-ray sensitivity[20]. Furthermore, when ^{125}I is incorporated into mammalian DNA, its decay induces a high percentage of DNA breaks that are not rejoined[21], whereas breaks induced by ^{3}H or X rays are rejoined[21,22]. ^{125}I has been shown to be more lethal per decay than ^{3}H and more lethal per rad than X rays[23]. Also, the size of daughter DNA synthesised in cells irradiated with either high- or low-LET radiations does not exceed the size of the parental DNA in up to 12 h of post-irradiation synthesis (our work, in preparation). This indicates that non-rejoining breaks in the parental DNA serve as permanent barriers to synthesis of daughter DNA and again suggests that non-rejoining DNA breaks are lethal lesions.

The alkaline sucrose gradient technique used here detects three classes of DNA damage as breaks: single-strand breaks, double-strand breaks, and alkali-labile lesions. Thus, it is not possible to determine the exact nature of the non-rejoining breaks detected in this study. But, a peaked response with LET for the induction of any test effect, such as we found for non-rejoining breaks, implies that two or more radiation–target interactions are required to induce the effect[24]. This type of induction kinetics is consistent with the induction of DNA double-strand breaks. There is also direct evidence that high-LET radiations induce double-strand breaks more efficiently in phage[25] and mammalian[26] DNA than do low-LET radiations. In addition, there is experimental evidence that non-rejoining DNA breaks induced by γ rays are actually double-strand breaks[29]. Thus, it seems likely that DNA double-strand breaks comprise at least part of the non-rejoining breaks seen here in alkali after high-LET radiations. Other possibilities should not be excluded, however. For example, it might not be

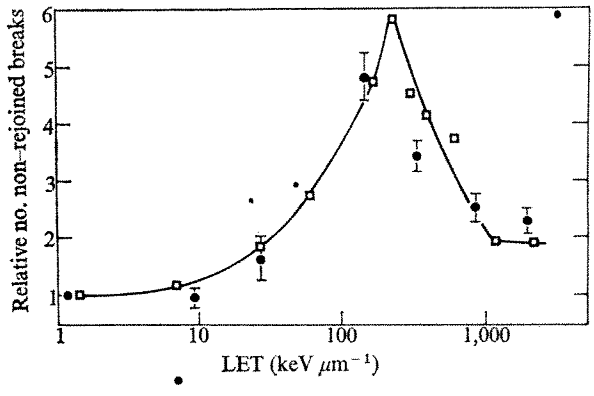

Fig. 2 ●, Relative efficiencies for non-rejoining break induction at various LETs: values obtained by combining results in Fig. 1*a* and *b* and normalising to the value obtained for X rays. ☐, Relative efficiencies for single-hit cell killing: these values were derived from data of Todd[18,19] for T-1 human kidney cells.

possible for intrastrand DNA breaks within a certain critical distance of each other to be repaired, or a break near some other lesion, such as a protein-DNA cross-link, might be inaccessible to repair enzymes. As is the case with double-strand breaks, high-LET radiations should induce these hypothetically non-repairable double lesions more efficiently than low-LET radiations.

Cell killing by ionising radiation can be characterised by two independent mechanisms having lethal, single-hit and sublethal, multi-hit attributes. Our results offer a molecular explanation of the lethal, single-hit component, relating it to non-rejoining DNA strand breaks. The identity of other lesions responsible for the sublethal component remains undefined, although it has been postulated that these lesions are rejoinable DNA single-strand breaks[17,29].

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Electrical demonstration of rapid light-induced conformational changes in bacteriorhodopsin

BACTERIORHODOPSIN (a purple pigment) is a light-driven proton pump[1,2], found in patches of the cell membrane of *Halobacterium halobium*[3,4], and is chemically similar to the visual pigment rhodopsin of animal eyes[4]. In both molecules the chromophore retinal is linked as a protonated Schiff base to a lysine residue of the apoprotein opsin[1,5,6]. Light induces bacteriorhodopsin to move protons from the inside to the outside of the cell. The resulting electrochemical proton gradient across the membrane is finally used to synthesise ATP. We report here the application of a new technique for the direct measurement of small capacitative charge displacements in oriented molecules. Our photoelectric measurements using asymmetrically aligned bacteriorhodopsin demonstrate that this protein undergoes reversible conformational changes after absorption of light.

Flash spectroscopy shows that bacteriorhodopsin undergoes a cycle through at least five different spectroscopically defined intermediates[7]. About 8 ms after bleaching the pigment returns to its original form. This cycling time is in good agreement with the maximal 'proton pumping' rate at saturating light intensities. Measurements show that a single protein molecule can transport up to 100–200 protons per second[8].

It is conceivable that the excited bacteriorhodopsin molecule, in addition, passes through a conformational transition sequence to accomplish the vectorial proton transport[9]. Such conformational changes are expected to generate charge displacements within the protein. To measure this effect electrically, two basic requirements have to be fulfilled. First, the molecules must be oriented in parallel but asymmetrically and second, they must be synchronisable, that is, they have to start with the change at a common zero time.

The most favourable system for fulfilling these conditions is found in photoreceptor cells of animals. Here, rhodopsin molecules are strongly oriented in lamellar cell structures, and synchronisation is achieved by means of short flashes. Fast capacitative photopotentials from a wide variety of photoreceptor cells have been measured, and have come to be known as 'early receptor potentials' (ERPs) (ref. 10). The signals are generally attributed to conformational changes within the rhodopsin molecule as it passes through its bleaching sequence[11].

We have succeeded in developing a model system in which rhodopsin from bovine retinal rod outer segments is reoriented on to one side of a thin Teflon film separating two aqueous phases[12]. Flashes to the layer evoke fast photovoltages and photocurrents (depending on the measuring device). Since the layer, having virtually infinite resistance when arranged between two aqueous phases, can be considered as a capacitor, the observed photoelectric response is due to pure capacitative charge displacements. Such model layers offer, for instance, the advantage of allowing the measurement of true transition time courses unaffected by cell membrane time constants. We have now applied this approach to bacteriorhodopsin layers.

Surface-active material from the aqueous suspension of purple membrane fragments was obtained by agitation until foam formed. About 100 µl of the foam was transferred to one side of a two-compartment Teflon cell containing a 4 M NaCl subphase. Thereafter it was overlaid with hexane. The surface film in the water–hexane interface was apposed to a vertically mounted Teflon septum (6 µm thick) by slowly increasing the water level. The chamber on the other side of the septum contained only electrolyte. Thus, the model system consists of the spatial sequence: electrolyte–bacteriorhodopsin–Teflon–electrolyte. The Teflon septum between the two half-cells acts as an electrical insulator and couples the compartments only capacitatively. The two identical electrolyte solutions were connected by silver–silver chloride electrodes to a high resistance input electrometer ($10^{14}\Omega$; 20 pF) (ref. 12).

The voltage responses of this layer when illuminated with bright white flashes are illustrated in Fig. 1. The individual oscilloscope pictures originate from sequential flashes to the same layer. They differ in the time axis to delineate the full time course of the signal.

Analysing the photopotential time course for exponentials leads to, at least, three clearly distinguishable transitions: the signal increases with a fast time constant $\tau_1 \sim 1$ ms and a slower one, $\tau_2 \sim 15$ ms. The main decay back to the initial potential occurs with $\tau_3 \sim 1$ s. Correlating these time constants to those of the spectral intermediates is not feasible at the present time and with the present technique. In spite of this, the close agreement of τ_2 with the 412 nm→570 nm transition ($\tau \sim 12$ ms) is striking. Also, millisecond spectral transitions have been observed which could coincide with τ_1 (ref. 9).

Since bacteriorhodopsin undergoes a cyclic photoreaction, the photovoltage would be expected to be reversible; and indeed it is. The bleaching cycle in the purple membrane is completed in approximately 10 ms, whereas in the model layer about a second is required. This discrepancy of two orders of magnitude is tentatively attributed to the different arrangement of the protein in the two systems: *in situ*, a lipid bilayer matrix, two sides of the protein contact aqueous media[13]; whereas *in vitro*, the protein is in contact with water only on one side—the more hydrophilic one. If a water-soluble reactant (presumably protons) is required for regeneration at the side where the bacteriorhodopsin molecule touches the Teflon surface, then the hindered accessibility would account for the inhibition of regeneration.

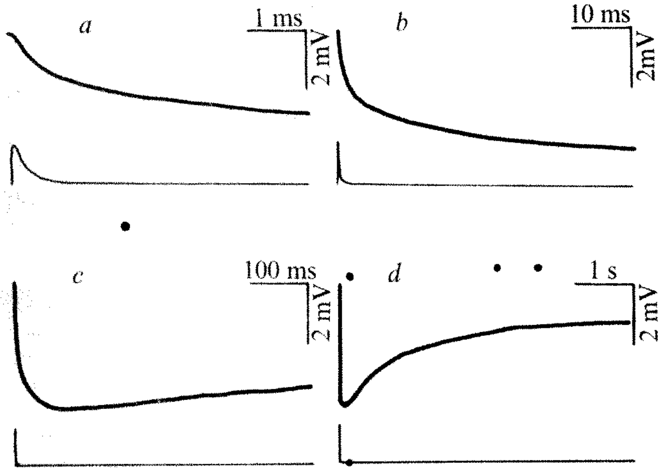

Fig. 1 Examples of the photovoltage of a bacteriorhodopsin layer, 6 mm in diameter, in response to white light flashes. Purple membrane fragments, prepared according to Oesterhelt and Stoeckenius[14], were suspended in 3.0 ml basal salt sodium 4.35 M NaCl, 0.275 M KCl, 0.08 M $MgSO_4$, 9 mM Na citrate, *p*H 7.0) at a protein concentration of 0.5 mg ml^{-1}. The aqueous subphase contained 4 M NaCl and 10 mM imidazole adjusted to *p*H 7.1 with HCl. The experiments were carried out at room temperature (22 ± 1 °C). Each oscilloscope trace includes the time course of the flash at the bottom. Subsequent flashes were separated by more than 1 min. The following amplifier time constants were chosen: *a*, 1 Hz–10 kHz; *b*, 0.1 Hz–3 kHz; *c*, d.c.–1 kHz; *d*, d.c.–0.1 kHz.

The polarity of the voltage is negative on that side where the layer is formed. This is the expected polarity for protons moving from the aqueous phase to the bacteriorhodopsin layer. If this were so, then a strong increase in photovoltage amplitude should result from reducing the ionic strength. Furthermore, the time constants recorded above should depend on proton concentration. Changing the electrolyte (while the layer remains apposed to the Teflon), from 4 M NaCl to 4 mM NaCl and then back to 4 M NaCl, however, did not alter the photovoltage amplitude nor the time constants τ_1, τ_2, τ_3, at any stage of the manipulations. Apart from stressing the stability of the bacteriorhodopsin layer this experiment rules out an origin of the photovoltage from a diffused ion double layer at the aqueous interface. More likely, the effect is based on charge or dipole reorientations in the bacteriorhodopsin molecule itself.

Changes of *p*H in the subphase at constant ionic strength did not significantly affect the time constants, τ_1 or τ_2 (range, *p*H 4–9). One effect of increased proton concentration is a faster decay of the photovoltage transient to zero. For instance, at *p*H 7, $\tau_3 = 2$ s and at *p*H 4, $\tau_3 = 0.5$ s. This gives further support to the above mentioned explanation for the slow regeneration rate: the increased proton concentration at lower *p*H compensates for the hydrophobic location of the hypothetical regeneration site.

At low *p*H the photovoltage amplitude increases slightly. A layer, which displays at *p*H 9 a maximal amplitude of 3 mV displays at *p*H 4 about 7 mV. At *p*H 3 the shape of the photovoltage response changes dramatically; the negative phase is reduced in its amplitude and a very fast positive transient of 3 mV appears. The whole signal has a duration of 200 ms. These experiments indicate a proton contribution to the generating mechanism; since this is already localised in the protein (independent of ionic strength), the photovoltage or a part of it may directly reflect a proton movement in the protein molecule.

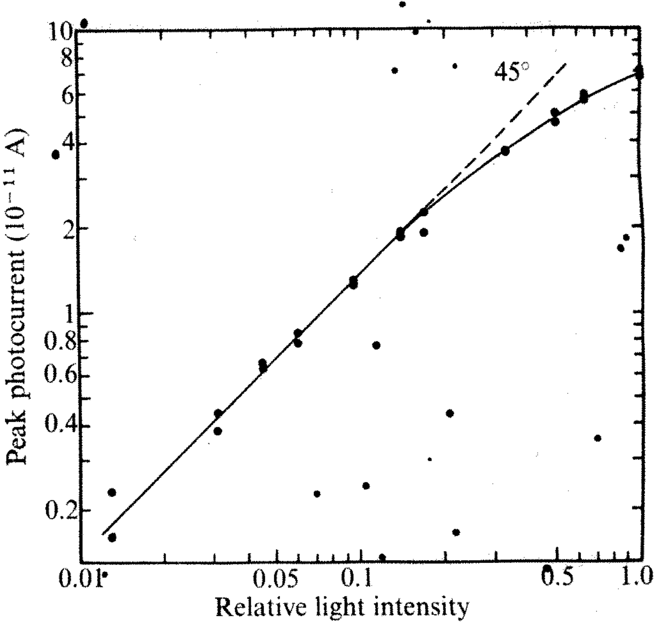

Fig. 2 Peak photocurrent as a function of the flash intensity. The increase in amplifier current time constant was limited to 1 ms. Neutral density filters were used to reduce the flash intensity. ● Data were obtained from one layer.

Since the bleaching of bacteriorhodopsin is reversible, it is possible to apply many flashes to the same layer, retrieving reproducible responses: one layer can be exposed a hundred times to a saturating flash without significant alterations of the photovoltage.

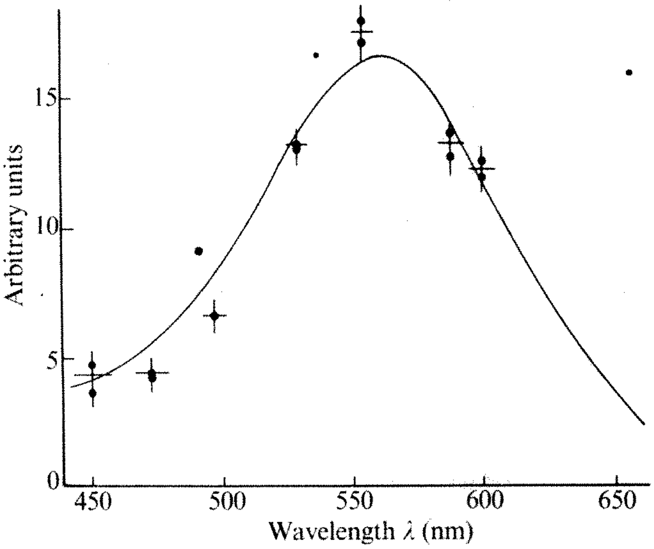

Fig. 3 Action spectrum of a bacteriorhodopsin layer. Solid line represents absorption spectrum of aqueous suspensions of bacteriorhodopsin. Each data point derives from the peak photocurrent at the given wavelength, normalised to the number of photons within the flash. Horizontal bars indicate the interference filter bandwidth at 50% transmission, and vertical bars represent the estimated experimental error.

The photoelectric effect can also be detected as a photocurrent if the electrodes are connected to an ammeter. In this case a photocurrent transient, which is exactly the first time derivative of the photovoltage, is measured. Therefore, the peak in the current transient reflects the steepest slope of the voltage. Since reducing the flash light intensity decreases the photovoltage amplitude, without affecting the shape, a dependence on light intensity could also be measured as the peak photocurrent amplitude. This method provides a better signal-to-noise ratio and is therefore used in the experiment illustrated in Fig. 2. For dim flashes the peak photocurrent is directly proportional to the light intensity. Brighter flashes lead to sublinear responses, indicating a saturation behaviour.

To further establish that the photoresponses result from bacteriorhodopsin bleaching, an action spectrum was obtained (Fig. 3), using the 'current' mode described above. The individual peak photocurrents lie without exception within the linear portion of the curve shown in Fig. 2. The absorption spectrum of the bacteriorhodopsin suspension used in the experiments (solid line) is fitted with its amplitude to the date points. The good agreement of both identifies bacteriorhodopsin as the molecular species responsible for the photoelectric effects.

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Catalytic effect of nitrosophenols on *N*-nitrosamine formation

THE effect of phenolic compounds on the rate of nitrosation of secondary amines is not a simple one. Tannins inhibit nitrosamine formation[1], but in suitable conditions gallic acid catalyses the nitrosation of diethylamine, the rate being dependent on *p*H and gallic acid concentration[2]. Some phenolic constituents of smoked foods[3] inhibit nitrosation of morpholine at *p*H 3.0. Phenolic compounds react with nitrite at a much faster rate than do secondary amines[4], but chlorogenic acid and 4-methylcatechol catalyse[5] the nitrosation of piperidine at gastric *p*H. We report here that nitrosophenols can catalyse *N*-nitrosamine formation.

Nitrosophenols are formed by the nitrosation of phenols and their presence in cured smoked meats has been demonstrated[6]. The reaction we studied was that of 0.15 M sodium nitrite with 0.15 M pyrrolidine in citrate buffer, *p*H 5.0, at 37 °C. Perchloric acid was used to bring the pyrrolidine solution to *p*H 5.0 before mixing. The effect of *p*-nitroso-*o*-cresol on this reaction was studied at 0.25, 0.50, 0.75 and 1.0 mM concentration using the method of initial rates. Aliquots were taken from the reaction mixtures at 5-min intervals for 20 min. Each aliquot was quenched with a solution of ammonium sulphamate in 2 N H_2SO_4 to remove unreacted nitrite, and the nitrosopyrrolidine (NPy) was extracted into dichloromethane and determined by gas–liquid chromatography. Each reaction was repeated in triplicate. For each reaction a plot of NPy concentration against time gave a straight line, the slope of which gave the rate of formation of NPy. Figure 1 shows a plot of the rate of NPy formation against nitrosocresol concentration. Figure 2 shows a plot of the logs of these two properties. That the points in Figs 1 and 2 do not fall exactly on straight lines may be due partially to the poor solubility of *p*-nitroso-*o*-cresol in the citrate buffer solution used. The slope of the line in Fig. 2 calculated by the method of least mean squares is 0.81, and this represents the order of the reaction with respect to *p*-nitroso-*o*-cresol. The two other nitrosophenols we have tested, *p*-nitrosothymol and *l*-nitroso-2-naphthol, also catalysed NPy formation at *p*H 5.0. No reaction takes place between *p*-nitroso-*o*-cresol and pyrrolidine at *p*H 5.0 in the absence of nitrite. The formation of NPy therefore does not occur simply by a trans-nitrosation process from nitrosocresol to pyrrolidine. We found that *N*-nitrosation does not occur in the organic phase during work-up by reduced nitrite species. Such nitrosamine formation requires the presence in the organic phase of nitrosating species and pyrrolidine. The acidic quenching procedure employed in these experiments, besides removing unreacted nitrite, will protonate unreacted amine. The protonated amine should remain in the aqueous layer during dichloromethane extraction. To check that this was so we prepared a 0.15 M solution

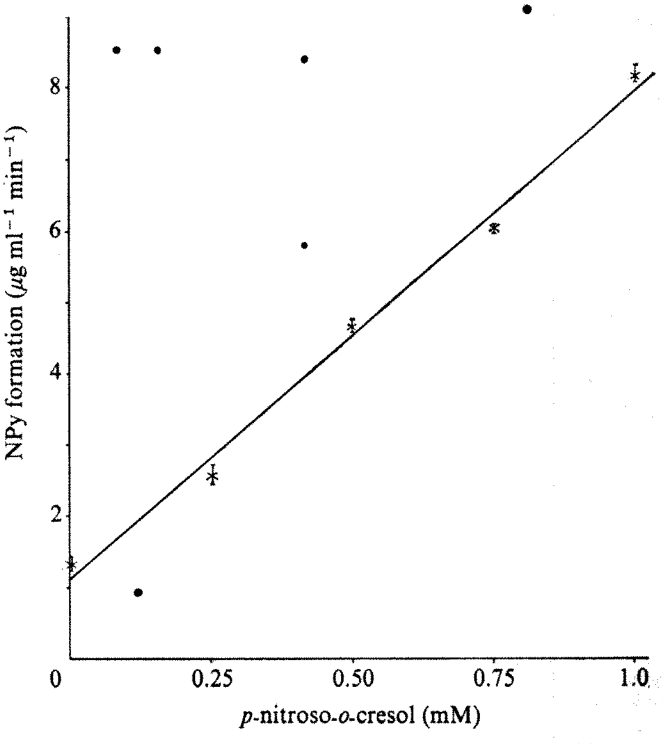

Fig. 1 Dependence of the rate of formation of *N*-nitrosopyrrolidine on the concentration of *p*-nitroso-*o*-cresol.

of pyrrolidine in pH 5.0 buffer, and put an aliquot through our quenching and extraction procedure. The organic extract was analysed for pyrrolidine in free and protonated forms. Free pyrrolidine was not detected, subject to a detection limit of 0.5 μg ml^{-1}. The level of total pyrrolidine (free plus protonated) detected in the extract was >1.0 μg ml^{-1}. The amount of NPy that could be formed from this is insignificant compared to the levels being detected in the reactions. In a reaction mixture containing sodium nitrite (0.15 M), pyrrolidine (0.15 M) and p-cresol (0.075 M) in citrate buffer at pH 5.0 the concentration of NPy formed after 1 h was two and half times greater than that formed in a control reaction from which p-cresol was absent. It therefore seems that at suitable concentrations the presence of phenols can lead to an enhancement of the rate of nitrosamine formation.

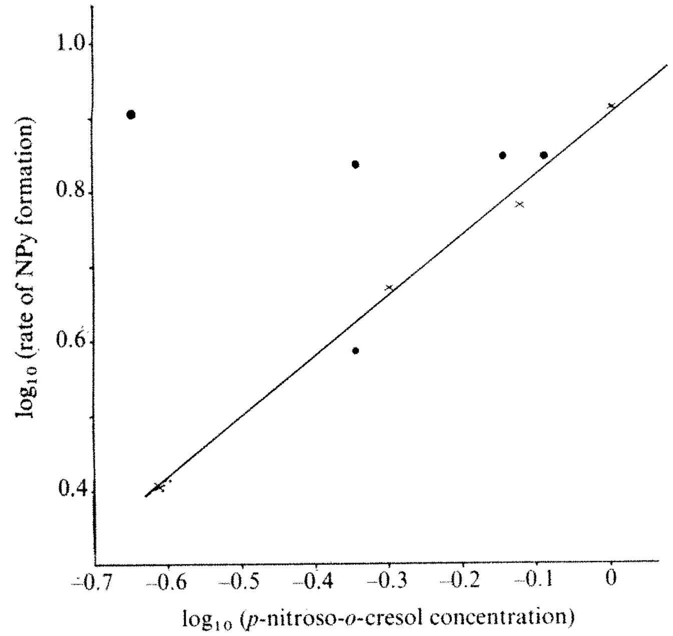

Fig. 2 Logarithms of the rates of formation of N-nitrosopyrrolidine plotted against the logarithms of the concentrations of p-nitroso-o-cresol.

Other kinetic data[4] suggest that in a system containing nitrite, a phenol and a secondary amine, the phenol should be nitrosated at a faster rate than the secondary amine. The initial product of phenol nitrosation is the nitrosophenol, a relatively unstable species, which can be oxidised by atmospheric oxygen to the stable nitrophenol. In this system there are therefore two opposing effects on the rate of nitrosamine formation. The rate will be retarded by the phenol, but catalysed by the unstable nitrosophenol formed during the reaction by nitrosation of the phenol. The overall effect is likely to depend upon whether the steady-state concentration of nitrosophenol (as determined by the relative rates of its formation and its destruction by oxidation to nitrophenol) is sufficiently large to overcome the inhibitory effect of the phenol. In turn this will depend upon the relative degrees of retardation and catalysis of nitrosamine formation exerted by the phenol and the nitrosophenol respectively.

These observations elucidate the basis for some of the conflicting reports of the effects of phenolic compounds on the rate of formation of nitrosoamines, but the implications in terms of the formation of nitrosamines in cured meats and *in vivo* are not clear. The effects of phenols at up to 300 mg kg^{-1}, which may be found in smoked meats (Knowles, M. E., unpublished) and of the 42–300 mg of phenolic material excreted daily into the stomach via gastric juice[7] are likely to be much more complex. The complicating effects of other soluble and insoluble components and of adsorption effects in heterogeneous systems are not susceptible to quantitative prediction; our studies of the catalytic effect of phenols which we have noted as being exerted through the formation of nitrosophenols are being extended to examine these additional factors.

We thank Mr Alan Young and Mr Colin Crews for technical assistance.

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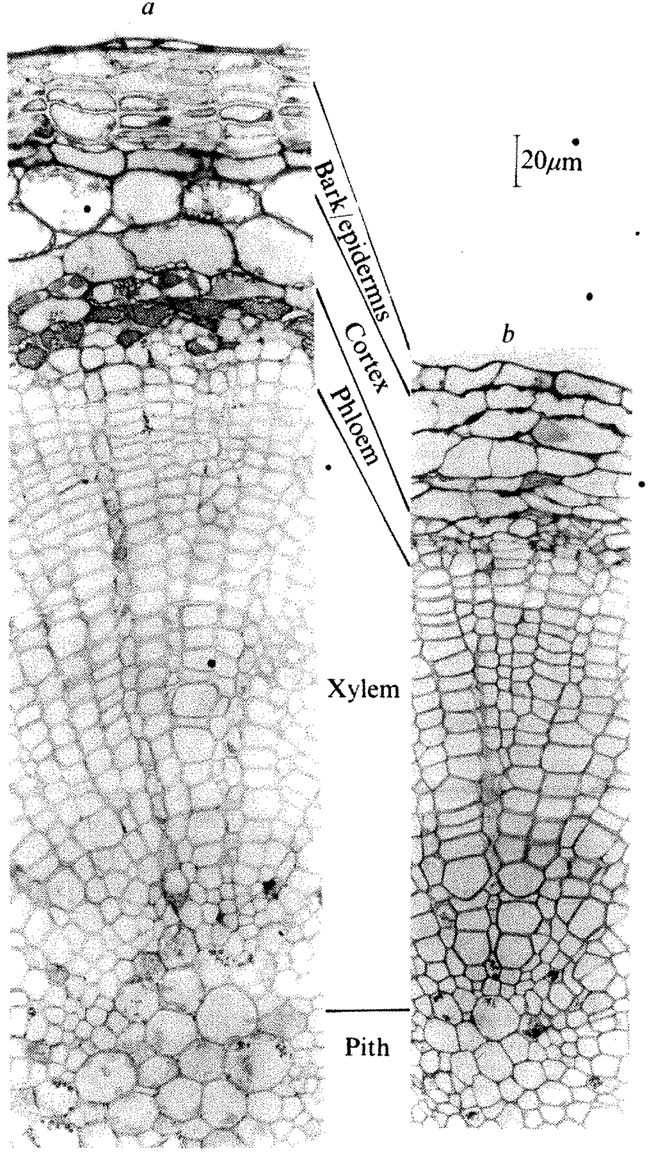
Erratum

In the article 'Stem anatomy and sheathing mycorrhizas in the *Betula verrucosa–Amanita muscaria* relationship' by P. A. Mason, J. Pelham and F. T. Last, *Nature* **265**, p. 334, Fig. 1*b* was inverted and is shown in correct relation to Fig. 1*a* below.



nature 21 April 1977

Carter's nuclear moves

THIS week, President Carter is set to unveil his much heralded and extensively leaked energy policy. He has been warning for weeks that its emphasis on conservation and its moves to increase energy prices will be politically unpopular, a fact vividly confirmed last weekend when, even before the policy was officially released, it came under attack in three separate television interviews given by Senator William Proxmire, the consumer advocate Ralph Nader and the Republican National Committee Chairman William Brock —people whose political philosophies span a wide spectrum.

Clearly, it will be a severe test of the fledgling Administration's political skills to push the energy proposals through a Congress which is all too aware that soaring energy prices may swiftly translate into plunging electoral votes. Nevertheless, Carter has suggested that he is willing to try, even at the expense of a few percentage points in his popularity rating.

While Carter's domestic energy proposals are running into heavy fire at home, his efforts to curb nuclear proliferation have been attracting equally fierce and often self-interested attacks abroad. The non-proliferation proposals, announced in advance of the other energy proposals, have been criticised unofficially by some West European governments who grumble that the United States may be trying to keep them out of a lucrative business in which they have a technological lead. And it has been attacked in some developing nations as an attempt by the United States to maintain the gap between rich and poor.

First, it is important to understand just what Carter did and did not say. There was nothing very surprising in his announcement, for Carter had signalled his punch months ago during campaign speeches and in some previous Presidential pronouncements. It was not a comprehensive, detailed discussion of major proposals. Instead, Carter simply stated several policy options which his Administration intends to pursue. They are:

- The United States will defer indefinitely the commercial reprocessing of fuel from power reactors and the recycling of plutonium. This means that no federal aid will be supplied to start up a reprocessing plant built by private industry in South Carolina, and that plant may well be mothballed before it even starts operating.
- The liquid metal fast breeder programme, which has previously enjoyed pride of place as the highest priority energy research and development programme in the United States, will be scaled down and restructured to give more emphasis to research and less to development of demonstration reactors. The programme will not be scrapped, however.
- More funds will be put into research on alternative nuclear fuel cycles, such as the thorium cycle, which may pose less risk of proliferation.
- The United States will increase its production capacity for enriched uranium in order to provide assurances to countries which agree not to develop nuclear weapons of adequate fuel supplies well into the future, the objective being to try to dissuade other nations from building their own enrichment and reprocessing plants to ensure their fuel supplies.
- The United States will continue to embargo export of equipment or technology that would permit enrichment or reprocessing by non-nuclear weapons states.
- Discussions with both nuclear exporters and importers will continue, and the United States is willing to explore various arrangements aimed at developing international fuel cycle facilities and access to spent fuel storage facilities for countries sharing non-proliferation objectives.

The only new policies announced are the renunciation of domestic reprocessing by the United States and the sharp reduction in efforts on the breeder reactor programme, both of which are important precursors to international non-proliferation talks. The proposal to defer domestic reprocessing was strongly urged recently by a committee established by the Ford Foundation, whose report seems to have provided the basis of Carter's policy. The reasoning, which is persuasive, is that if the United States pursues domestic reprocessing while trying to dissuade others from doing so, it would be accused of setting a double standard and its credibility would be destroyed. Moreover, since the United States has relatively large coal and uranium resources, the incentive to reprocess is small, at best.

Carter quickly acknowledged that other industrial countries might see their own domestic programmes in a different light, since their future energy supplies might not be so stable as those of the United States. Nevertheless, the onus is now on those countries which intend to reprocess to discuss publicly the reasons which compel them to push ahead at the possible expense of some lessening of control over proliferation.

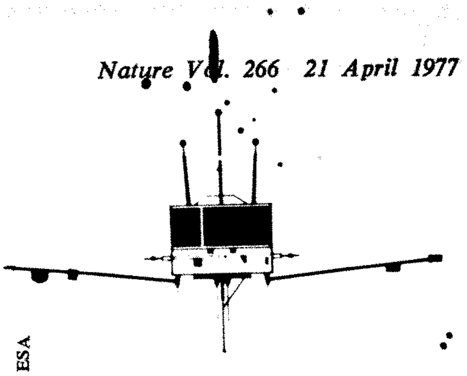
The next opportunity to discuss these matters in an international forum will be at the secret talks between nuclear suppliers which are due to resume in London in the next few weeks. Carter's statement, released a month before the talks are scheduled to resume, has set the stage for the talks and it has provided plenty of opportunity for a more thoughtful response by European governments than the mostly self-serving reactions which have been voiced unofficially so far. ☐

Nature Vol. 266 21 April 1977

Geos

Europe's satellite for science

Geos, the ESA's purely scientific satellite, is launched this week. **Stuart Sharrock**, now in Florida, reports


ESA

THE EARTH lies inside a leaky magnetic bottle which is formed by magnetic field lines linked to the earth. Called the magnetosphere, it stops the blast of particles from the sun penetrating to the ground and prevents the escape of ionised particles from the upper atmosphere. Occasionally leaks occur, producing aurora and terrestrial magnetic storms that play havoc with radio communication and electrical power distribution. But more significant is the role the magnetosphere plays in controlling the earth's climate. Solar–climate correlations are well established and although it is clear that the magnetosphere mediates between the solar wind and the atmosphere, the exact mechanisms by which it does so are not understood in spite of the several hundred satellite missions already flown.

Study of the magnetosphere is clearly important, for it should lead to an understanding of the environment which will bring economic benefits; but there are other reasons for investigating its properties. Ninety nine per cent. of all matter in the universe is in a plasma state interacting with a magnetic field and the process by which magnetic field energy is transferred to ionised particles is of fundamental significance. This process occurs in the magnetosphere, which is therefore a unique laboratory—a private universe in our own backyard—in which we can study small-scale versions of the fundamental mechanisms that govern the behaviour of matter in the universe. Potential prizes from probing the magnetosphere range from more accurate weather predictions to a better knowledge of the nature of pulsars; from an understanding of aurora to improved prospects for controlled nuclear fusion. The basic structure and processes of the magnetosphere have now been identified and an attack has been launched on the detailed mechanisms and the dynamics of the system as a whole. It is known as the International Magnetospheric Study (IMS) and will run from 1976–79.

Two major satellite missions are at the heart of the IMS: the world's first purely scientific geostationary satellite, ESA's Geos, and a combined NASA/ESA venture comprising three satellites code-named ISEE (International Sun Earth Explorer). Geos is scheduled to be launched on 20 April and ISEE on 14 October; as the centrepiece of the IMS, Geos has been designated the reference spacecraft for the study and will coordinate with ground-based, balloon, aircraft and rocket programmes.

The magnetosphere

A solar wind of protons and electrons blows continuously through the solar system at some 400 km s^{-1} and, according to Alfven's 'frozen-in' theorem, drags with it the Sun's magnetic field. The earth's dipolar magnetic field and the ionospheric plasma are similarly frozen together. These two magnetic fields cannot mix and so the earth's field lies in a closed cavity in the plasma stream from the Sun and presents a tiny obstacle to the blast of the solar wind. This magnetic bubble is the magnetosphere; it is deformed from a dipolar configuration by the solar wind and the size of the magnetospheric cavity changes in response to the strength of the wind. At typically 10 earth radii the hot plasma from the Sun is deflected and streams around the earth's protecting magnetic bottle; under certain conditions however the bottle becomes open to the solar wind —with drastic consequences. Within the magnetosphere there are numerous plasma regions which respond to changes in the solar wind and which interact with the ionosphere and the atmosphere.

Observations with a single satellite can never distinguish unambiguously between spatial and temporal variations; two satellites orbiting together with a known separation are needed and this is one of the aims of the ISEE mission. Geos will sample the different regions of the magnetosphere, measure minute variations in all the particle and wave parameters without interference from the satellite itself, monitor conditions continuously over many months, and yet be capable of responding to changing situations in minutes, and explore the coupling between the magnetosphere and the ionosphere by enabling correlated satellite and ground-based measurements. These requirements specify a satellite with an orbit, construction and performance an order of magnitude more complex and sophisticated than any previous ESA satellite and with a control and ground support system more ambitious than has so far been attempted by NASA with unmanned missions.

The Geos satellite

Built by a consortium of European industrial contractors headed by the British Aircraft Corporation, Bristol, England, Geos will be placed into a geostationary orbit 36,000 km above the equator. The orbit covers a region of the magnetosphere where many of the dynamic processes responsible for magnetic and ionospheric disturbances are believed to develop; the satellite will be positioned at longitudes where the magnetic field lines are conjugated to both auroral zones (that is, particles passing along the field lines through the satellite will arrive at the aurora), allowing correlated measurements to be made from ground stations and rocket-borne instruments.

As the spatial variations seen by Geos will be small, the time and space ambiguities will be minimal and short-lived transient phenomena can be studied. Sampling of different regions of the magnetosphere will be automatic as Geos alternates between the day-side and the night-side, but the boundaries of the different magnetospheric regions are not fixed in space and will at times move across the geostationary orbit under the influence of the solar wind; phenomena associated with such boundary crossings are of particular interest.

Once in orbit, Geos will be permanently in view of a purpose-built tracking and data recovery ground station near Darmstadt in West Germany (also to be used for Meteosat). The continuous link between the satellite and the ground allows for high data rates, which are essential for some experiments, and means that real time modification of experiments becomes possible. The capability of closed loop control of instrument modes is crucial and was laid down as a ground rule for the mission in the early planning stages.

The problem with many satellite experiments is that effects caused by the spacecraft itself can often mask or destroy the phenomena of interest. Spacecraft charging is well known—

potentials of up to 10 kV have been reported from the ATS satellites during eclipses—and can have disastrous effects on experiments to measure low energy particle fluxes. To minimise this problem the whole of the outer skin of Geos has been made conducting, thus ensuring that the surface is an equipotential. There is a complex system of eight booms which deploy in orbit to take the sensitive detectors up to 20 m away from the disturbing influence of the satellite body; this is the most complex boom system attempted for any European satellite. The experiments mounted on Geos involve extremely sensitive measurements (for example the detection of magnetic variations of a thousand millionth of the strength of the earth's magnetic field) and this has meant an extensive programme to minimise electromagnetic interference caused by equipment on the spacecraft.

The experiments

The achievement of providing a magnetospheric laboratory free from interference, accessible in real time and controllable from the ground is matched by the scope of the experiments comprising the payload. To understand the magnetosphere's structure and the wave–particle interactions that occur, it is essential to make sensitive measurements of all relevant parameters simultaneously. For the first time this is being done and data on every quantity of interest are being collected: four experiments will measure particle fluxes and their variation with energy and direction, covering a range from thermal energies to hundreds of keV, and three experiments will measure electromagnetic waves over a frequency range 0–77 kHz for electric fields and 0–20 kHz for magnetic fields. DC electric and magnetic fields will be monitored with extreme sensitivity. The most important measurements will be duplicated by independent experiments.

A mission this complex cannot hope to satisfy all the requirements of a number of coordinated campaigns; many of the needs are mutually exclusive and inevitably compromises must be made. The coordination of activities is crucial to the success of the mission and has been an important factor in its planning. Areas in which the standard configuration is not ideal can hopefully be covered by special arrangements; the project has the inherent flexibility to allow this.

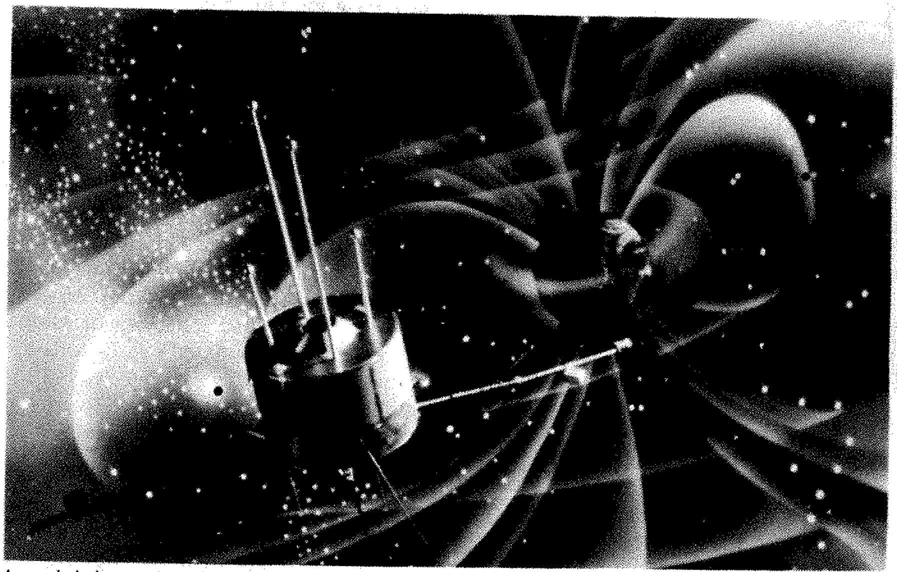
As an example, a polar expedition to a ground station in the Arctic could be unhappy about the orientation of Geos. The expedition will want to correlate its measurements with particle fluxes coming from the south at the position of Geos but this is only possible during the northern winter. The directional coverage for particles is not isotropic and the orientation of Geos is governed by the requirement that the radial booms do not shadow the solar cells. Inversion of the satellite —a delicate operation—would have to be arranged but it would interfere with the wave experiments and expeditions to the Antarctic would not be too happy. Other ground stations would no doubt have conflicting requirements. Rocket campaigns, for example, are costly and can only be conducted at certain times, and these are sure to be the times when interesting transient effects are manifesting themselves and necessitating an immediate change in plan. In any case there is only a limited supply of fuel so manoeuvres are rationed. The coordinating committee will have a tough time but the number of demands made on the mission is a measure of its flexibility and importance; the number of satisfied customers is likely to be very high. Crucial to this flexibility is the provision of extensive computing facilities at the ground station where the data are collected.

Coping with data

Most of the time nothing exciting will happen (the magnetosphere can remain stable for long periods) and it is unwise to accumulate vast quantities of data throughout the mission. Filling a magnetic tape every 45 minutes, Geos would generate 26,000 tapes over a two year period of which only about 10% would be worth keeping. The solution is to recycle the magnetic tapes every three months; there is an extensive data distribution system (it includes daily summaries of all Geos data) to enable a communal decision on which data are to be retained. Not only does Geos have the ability to respond quickly to changes in the magnetospheric system, it also imposes a requirement on the experimenters to respond to their data within a reasonable time period—a valuable constraint that could be more widely adopted.

Originally planned for August 1976, the launch of Geos has been delayed until April 1977 mainly because of late delivery of components. The satellite will be launched by a NASA Delta launch vehicle from the Kennedy Space Center in Florida. The original flight plan has been continually modified to take account of the magnetic conjugacies between a given longitudinal position of the satellite and the positions of available ground stations and rocket campaigns. The current plan is to station it initially at a longitude of 0°, where a double magnetic conjugacy exists between Iceland in the northern hemisphere and the Syowa base in the southern hemisphere. In about August this year Geos will be drifted eastwards to arrive at about 35° E by October 1977; this is a position ideally suited for correlated measurements with the extensive array of ground-based stations and rocket ranges in Northern Scandinavia. In mid-1978 the satellite could be thrust back to the 0° position again, further movements being a function of the status of polar expeditions, rocket campaigns, ground stations, the amount of fuel remaining, the consensus of opinion and the tempers of the Coordinating Committee.

After lift-off it will take about a month to get Geos into its correct orbit with the extensive boom system correctly deployed, another month to check out the experimental payload; by then hopefully it will be fully operational and the IMS can start in earnest. The taxpayers of Europe have made a wise investment in Geos—if NASA gets it up there. ☐



ESA

An artist's impression of Geos in the magnetosphere

A partial triumph

Vera Rich assesses the Moscow Seminar, which celebrates its fifth anniversary this month

THE illicit Moscow 'Sunday Seminar' for refusnik scientists was founded five years ago this month. In a remarkable development after such a lengthy period of harassment of its organisers and participants, the Soviet authorities have now informed Professor Mark Azbel, the current leader of the seminar, that he may hold a special anniversary meeting this week provided it is confined to matters of strictly scientific interest.

The first Sunday seminar for refusnik scientists—that is, Jewish scientists dismissed from their posts and deprived of all official means of pursuing their careers—took place in Moscow on 12 April 1972. The date is significant— 1972 was the year of the 'education tax', by which any Jew wishing to emigrate to Israel was obliged to repay to the government the 'cost' of any higher education he may have received. And since such education is provided *gratis* by the State, the authorities could clearly fix its notional cost at any value they chose.

Scientists wishing to emigrate became liable to long waiting periods between applying for a visa and receiving permission to emigrate. They therefore began to form one of the most significant social groups within the growing class of *otkazniki* (the word which later became transformed in English to 'refusnik')—those not permitted to leave the country, yet deprived of their professional means of livelihood within it.

Moscow meeting

Against this background, a modest group of 17 scientists met in the Moscow flat of Dr Henrikh Sokolnik in April 1972. The venue was chosen because Dr Sokolnik is a chair-bound cripple. Their aim was to read papers of mutual interest and thus to keep up the interplay of ideas without which scientists cannot function professionally. Throughout 1972 the group functioned quietly without attracting the attention either of the Soviet authorities or of Western sympathisers. Even the weekly Bulletin *Jews in the USSR*, founded that year to publicise the plight of refusniks and Jewish prisoners of conscience, made no mention of the seminar, although it carried numerous appeals from scientists to their colleagues abroad, and news items about scientists suffering particular hardship (notably Dr Evgenii Levich, who in that year was sent to a Siberian labour camp).

It was not until 1973 that the first information about the seminar began to reach the outside world. In that summer, when an International Conference on Magnetism was meeting in Moscow, several Western scientists, learning of the exclusion of the refusniks from the conference, made their way to the flat of Professor Aleksandr Voronel, where the seminar was then meeting, and took part in a lively and informative discussion. From this contact the idea arose of holding an International Session of the seminar the following summer.

The International Session was widely announced. In addition to Professor Voronel, two organising secretaries, Dr Norman Chigier of the University of Sheffield and Dr Edward Stern of the University of Washington, Seattle, were appointed. Papers were invited on an international basis, and more than 50 leading scientists, including seven Nobel prize-winners, lent their names, either as patrons or as would-be contributors. The preparations caused considerable disquiet to the Soviet authorities which, in the stress of the situation, made what can be seen in retrospect as a tactical mistake.

Had the seminar been permitted to take place, then coming as it did at the height of the scientific conference season (July) and virtually coinciding with President Nixon's visit to Washington, it might have merited at best a paragraph in the foreign press as a promising sign of a new 'thaw'. Instead, prospective Western visitors were denied entry visas, the proposed venue was put under KGB supervision, the Moscow organisers were arrested and taken to detention centres outside the city, and other would-be participants were kept under virtual house arrest. The 'Seminar-that-never-was' accordingly went into history as a major example of State interference in academic freedom, and the 'Voronel Sunday Seminar' became an important (if unofficial) item on the schedule of scientists visiting Moscow in the course of their professional duties. It is estimated that, to date, some 150–200 foreign visitors have attended the Seminar.

Remarkable survival

The survival of the seminar over five years is, under Soviet conditions, remarkable. Of all those who took part in the first meeting only one is still in Moscow: Corresponding-Academician Veniamin Levich. The others, one by one, and largely due to pressure from their Western colleagues, have received their permission to leave. Professor Voronel himself received an exit visa some six months after the non-occurrence of the International Session, and his role as leader has been inherited by Dr Mark Azbel, with, in recent months, Dr Anatolii Shcharanskii acting as press attaché and contact with the international scientific community.

As the more 'senior' members of the group leave, there are always new refusniks to fill their places, and there seems little likelihood that the need for a seminar will disappear within the foreseeable future. Indeed, the seminar movement has proliferated: by last summer some ten additional seminars had been established dealing with specialised topics, including theoretical physics, computer science, radio-electronics, and biology; and one seminar, in Kiev, after being officially 'closed' by the KGB, began meeting in a public park. Organisers and participants from all groups have experienced constant harassment including police interrogation, confiscation of books and papers, and short-term arrests.

One reason for the Moscow Seminar's survival is undoubtedly the interest shown by many scientists abroad. For them, the existence of a clear-cut ethical issue is involved: the UN Declaration on Human Rights states unequivocally that everyone has the right to leave any country, including his own. But in practice a number of caveats arise as far as scientists are concerned which makes the right difficult to exercise. Access to classified information is one objection; another, which in the case of the Soviet Union gave rise to the notorious 'education tax', is the brain drain with all its attendant questions of how far a student, in accepting a place for higher education, may be deemed to have implicitly entered into a contractual obligation to work in the country which has financed him.

Scientists remain shocked, however, at the idea of their colleagues being prevented from functioning as scientists. The holding of seminars and discussions, access to new publications and correspondence and meetings with foreign colleagues, all constitute a major part of the scientist's basic activity. Even if the Soviet authorities see fit to dismiss from their professional posts scientists so 'disloyal' as to wish to emigrate, and to restrain that emigration in the interests of the State, it seems illogical then to prevent them exercising, even unofficially, that profession which makes them a State asset. The right of the refusniks to hold seminars must appear unequivocal, even to those of their foreign colleagues who are disinclined to enter into long arguments against the Soviet government's pragmatic case for denying exit visas.

Contribution not great

But what, scientifically, is the value of these seminars? From the point of view of the progress of world science, it must be admitted that the Soviet contribution is not great. A handful of papers by refusniks has been accepted by foreign journals, but it is significant that these are by the more mature members of the group—notably Veniamin Fain, Mark Azbel and Viktor and Irina Brailovskii. The papers are, presumably, the working-up of ideas which the authors had no time to develop during their active careers, and for young scientists, who have not yet developed such a back-log of topics, the prospect of publication abroad is probably somewhat bleak.

Nevertheless, from the point of view of the scientists themselves, attendance at the seminar is something more than a holding operation. Soviet science, aimed as it is as serving the national economy, can be extremely narrow, so that one hears of first degrees in such topics as light metal engineering for the civil aviation industry. Moreover, theoretically Soviet science must serve socialism, and every working scientist must, officially at least, formally confirm his political commitment.

In the Seminar all this is changed. Thrown out of their jobs, the refusniks found a new freedom in their release from this tacit agreement to serve political ends. "We stopped mourning for our scientific interests, since, in truth, we had attained the ideal of scientific detachment", Voronel explains in his preface to the 'Proceedings' of the Seminar-that-never-was. At the very first meeting, Levich joked that "This reminds me of my youth. Back in a group of Jews again, and once again they talk nothing but science".

Over the years, this first flush of euphoria has somewhat faded, but the broadening of the participants' interests has not. Asked last week to comment on the achievements of the Seminar during its five years' existence, Professor Voronel remarked:

> "The main difficulty of the seminar has been to combine the different interests of its participants, but it can also be an advantage because they become aware of different fields. This may be of great value to contemporary science, to have such an interdisciplinary forum."

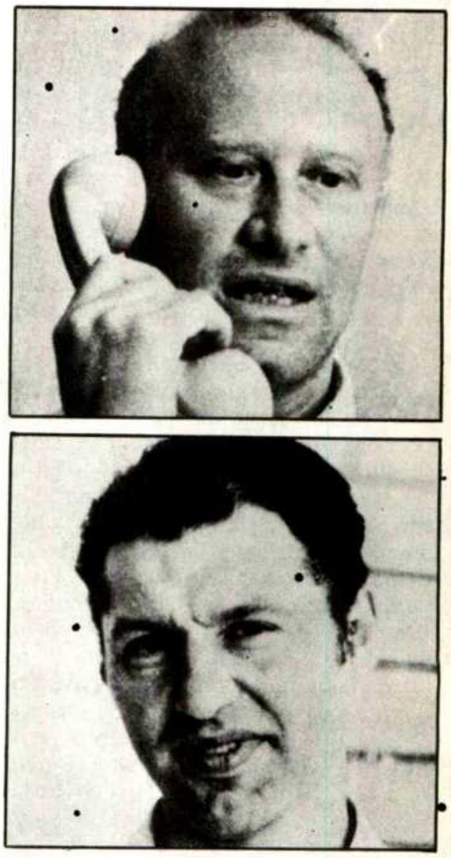
He cited the case of Shepelev, who from a discussion of general principles of phase transitions developed a new approach to his own subject—the strength of rubber and polymers. He also spoke of Roginskii, originally a nuclear physicist, who during the course of the seminars became interested in solid state physics and, on receiving his visa, was able to take up a post at the Hebrew University, Jerusalem, working in this new field.

This broadening of intellectual interests is, of course, considerably restricted by the lack of access to published literature, particularly from abroad. One project in particular, a sociological study for which surveys and questionnaires were distributed under conditions of great difficulty, would have had considerably greater intrinsic value had comparable figures, published during the past few years in the USA, been available for comparison. Foreign scientists have been invaluable as a source of information on the main trends of research abroad, as well as a profound source of moral support.

How much easier?

The fact that the authorities have given permission to hold the one meeting (although, strictly speaking, no such permission is required, since the Soviet constitution guarantees the right of assembly) will not necessarily mean that there will be no interference with the regular Sunday meetings. And, even if these can proceed without harassment, access to published literature will still be restricted to those few members who have retained their right of entry to academic libraries, and to the few hand-written copies of the primary journals available as *samizdat*. Experimental facilities will be lacking, and although the Russian term *nauka* covers the humanities and social sciences as well as the physical sciences, it seems unlikely that the authorities would look with favour on any further attempts to make sociological surveys; the data from the first such survey was confiscated last December.

Any attempts to hold another International Session, as opposed to welcoming visitors on an *ad hoc* basis, would doubtless still be interpreted as a 'foreign provocation', as it was in 1974. Furthermore, Anatolii Shcharanskii, the Seminar 'spokesman', is still in custody, facing the possibility of treason charges being brought against him; and within the past few months several members of the Seminar have been accused in *Izvestiya* of being agents of the CIA. Even if permission were now to be extended to the regular meetings of the Seminar, this would be of little value if it were to be accompanied by harassment of individual participants on grounds other than their membership.



Azbel (top) and Voronel: activists

The granting of the permission is nonetheless of far wider significance than a graduging acquiescence in the holding of a one-off celebration. Just as, in the words of Professor John Ziman, the true value of the Seminar "is not the work they are doing but the fact that they are doing it", so too the significance of this permission is not that the Soviet authorities will permit one special meeting of the Seminar to be held, but the fact that they admit the Seminar exists at all. In 1974 foreign scientists wishing to attend the International Session were refused visas on the grounds that they had not been invited by a *bona fide* organisation. In December 1976, when the Seminar group was to hold a special symposium on Jewish life in the USSR, the leaders were arrested and their right to hold the symposium was emphatically denied. Throughout the past five years, the authorities have done their best to deny the existence of the Seminar, and proceedings against its members and activitists have been brought on other counts, such as 'parasitism' (being without visible means of support) or 'slandering the State'. The granting of permission even for one meeting represents a real achievement, not least for science. ☐

Geophysics stakes its claim

A draft report proposing a massive deep sea research programme is now circulating among US earth scientists. Colin Norman reports from Washington

A PROPOSAL for an ambitious deep sea research programme, costing some $400 million over the next decade, is being put together by a group of earth scientists and will soon be forwarded to the National Science Foundation (NSF) for further study and possible funding. The proposal, based largely on recommendations developed during an international meeting held last month at Woods Hole, Massachusetts, would involve extensive drilling deep into the ocean floor in regions which have not yet been punctured by research probes.

A central feature of the proposal is to convert the infamous *Glomar Explorer,* the vessel used by the Central Intelligence Agency in 1974 to scoop bits of a sunken Soviet submarine from the bottom of the Pacific, into a sophisticated drilling ship. The *Explorer,* which is now in mothballs near San Francisco awaiting a user, would be capable of drilling at depths and in regions which are beyond the reach of present drilling vessels.

Though it is far from certain that the proposal will be funded, at least in its present form, it is sure to attract considerable attention as it wends its way through the federal bureaucracy. And the attention will not be confined to the United States, for NSF is expected to seek international participation in the venture if it decides to apply to Congress for funds.

A draft report outlining the proposal is now being circulated among a number of earth scientists, and a copy has been made available to *Nature.* The proposal would involve a major expansion and re-direction of the Deep Sea Drilling Project (DSDP), a programme begun in 1968 which has resulted in the drilling of more than four hundred holes in the ocean floor in many parts of the world. DSDP now runs an international effort called IPOD1—the International Phase of Ocean Drilling—which is supported by six different countries (the United States, which provides the bulk of the funds, France, Germany, Japan, the USSR and the UK). It has helped to revolutionise thinking about the history of the earth, but after several extensions it is due to expire in September 1979.

The new proposal is thus an attempt to map out a research effort to carry on where IPOD1 is expected to leave off. Drafted by an international committee headed by Manik Talwani of the Lamont-Doherty Geological Observatory, the proposal is the result of an elaborate planning effort involving scores of earth scientists. The committee was established last August by the executive council of JOIDES (Joint Oceanographic Institutes for Deep Earth Sampling)—a collection of research institutes which are taking part in the DSDP—and it was given a broad mandate to plan a research effort stretching over the next decade.

The committee's proposals are based on a series of 'white papers' drawn up by groups of scientists and discussed at the Woods Hole meeting last month. Its report has been approved in principle by the JOIDES executive council, and it is now in the final stages of revision before being published.

The DSDP has already provided masses of information confirming and refining the theory of plate tectonics, according to which the continents were once joined together in larger landmasses which were split apart by processes which still push the continents around. Those processes, known as sea floor spreading, involve the continual generation of new oceanic crust along the mid-ocean ridges where molten lava wells up from the mantle, solidifies and is pushed aside by yet newer lava. This constant generation of new crust gradually forces continents apart and is responsible for a variety of geophysical and geological phenomena, such as earthquakes, volcanoes, and large scale uplift of mineral deposits in many regions where oceanic and continental crusts converge.

Though rock samples recovered from many parts of the world by the DSDP drilling vessel, the *Glomar Challenger,* have produced solid evidence in support of the plate tectonic model, a number of intriguing questions remain (see also *Nature,* **259,** 83). They include a satisfactory explanation of why the continents split apart when they did, a better understanding of the active processes taking place along the oceanic–continental margins and an appreciation of the large-scale climatic and oceanographic changes which have accompanied the formation and evolution of the oceans. Many of those questions could only be answered by drilling into the ocean floor in regions which are at present inaccessible to the *Glomar Challenger.*

In particular, some of the most intriguing drill sites lie along the continental margins in regions where there is a risk of encountering oil and gas deposits, or deep in the ocean trenches where very long drill strings would be required, and at high latitudes where rough seas and ice present problems. A ship capable of coping with those conditions would require massive lifting capacity to handle the heavy equipment needed to prevent blowouts of oil and gas and to operate long drill strings. It would also require powerful engines, a thick hull and good station-keeping ability to work at high latitudes. Such a vessel would cost about $100 million to build, according to NSF estimates, a price tag which probably puts it out of reach. That, however, is where *Glomar Explorer* may come in.

Built for the CIA by Global Marine Inc., the same company which owns and operates the *Glomar Challenger,* the *Explorer* has been idle since 1975 when its mission became publicly known. Though it belongs officially to the federal government, a number of attempts to find a user for the vessel have so far drawn a blank. Last year, however, NSF expressed interest and commissioned Global Marine to study the feasibility of converting the ship into a deep sea drilling vessel. The study, which has recently been completed, concludes that it would cost about $52 million to convert the *Explorer* into a sophisticated drilling vessel, equipped with a riser to prevent blowouts if oil and gas are struck. The cost of operating such a vessel would be about $33,000 per day, about twice that of the *Glomar Challenger.* In other words, the *Explorer* could provide a cutprice entry into the more intriguing drill sites, and the Talwani committee envisages it becoming the principal actor in a deep sea research programme in the late 1980s.

The proposed programme would essentially involve continuing use of the *Glomar Challenger* to drill relatively shallow boreholes in accessible sites until October 1984. In the meantime, the *Explorer* would be converted in time to start drilling in 1981. It is anticipated that the programme would run until October 1987, and the total cost, including preliminary surveys and sample analysis but excluding the cost of converting the *Explorer,* would be about $396 million.

The proposed effort would include drilling in the passive margins—the continental margins which originally fitted together—in an effort to understand the processes which led to the original fusion of the super-continent. Another major thrust would be to drill along the active margins where the Earth's crust is being jostled and subsumed into the mantle, in an attempt to understand some of the geophysical processes involved. Other features of the proposal involve extensive drilling into the ocean crust itself in an effort to learn more about the processes by which it was formed, and to study the evolution and environmental changes which have occurred.

The committee cautions that the programme should not be attempted at all unless there is a firm assurance of support for extensive geophysical survey work before drilling takes place, and sufficient funds to analyse and interpret the results. "An investment in a drilling vessel without a commensurate investment in scientific investigations on an appropriate scale could not be supported in good conscience", the report states.

The proposal will be sent officially to NSF in the next few weeks, and responsible programme officials will seek authority from the Director and the National Science Board to look further into the scientific merits of the plan (a task which, as one NSF official wryly observed last week, will be complicated by the fact that virtually everybody capable of offering an independent opinion participated in drafting the proposal). If the programme is deemed meritorious, NSF will eventually seek funding, first from the Office of Management and Budget and later from Congress. At some stage, it is almost certain that the matter will land on the desk of Frank Press, President Carter's science adviser, who is a distinguished geophysicist himself.

For a project of that cost and scope, international participation would be an obvious advantage. At present, non-US participants in the DSDP each contribute $1 million a year in direct costs and a further sum in supporting the work of their own researchers in analysing and interpreting the results. NSF officials note that economic and political difficulties might mitigate against foreign participation.

The British commitment, for instance, is to support IPOD1 until 1978, and a request for a further year's support is expected soon. The British package of support is complex, however, involving sums of money from three separate departments, and any request for financial commitment at this stage for a period of ten years might pose problems.

The first decade of drilling with the *Glomar Challenger* has provided some spectacular results and answered some fundamental problems. But it has also thrown out some intriguing questions which can be answered only with more expensive equipment which will be more costly to operate. The same pattern can be discerned in fields such as particle physics and even molecular biology. It should be no surprise that it is now happening to geophysics. ☐

NETHERLANDS

Dutch go ahead on DNA

Casper Schuuring reports from the Netherlands on moves to control recombinant DNA research there

RECOMBINANT DNA research should be developed in the Netherlands because of its great scientific importance, according to a report from the country's Commission on Genetic Engineering which was handed over to the Ministers of Health and for Science Policy on 3 March and published on 30 March. The research should be carried out with stringent precautions and according to clear regulations contained in legislation which, in the commission's opinion, should be implemented quickly.

The commission, which started work just one year ago, also proposes a special committee with responsibility for surveillance of the research. It would continue the work of the present commission on a legal basis, and have on it representatives of the biomedical sciences, of other related sciences (legal and ethics), of the government and of society at large. The commission, which consists only of experts in the field, also suggests another committee to consider broader ethical and social aspects of the applications of advanced medical and biological research and whether to bring such research within the framework of legislation.

The commission supports the European Science Foundation's recommendation that the rules of the British Williams report be followed, but considers that on a couple of points the report is insufficiently clear. Plans for recombinant DNA research must be reported to the 'surveillance committee', and research cannot go ahead unless a statement that there is no objection to it has been obtained and an 'examination commission' has been established to check that all requirements are fulfilled. That body has the right to do investigations on the spot.

The commission prefers the description of the four levels of physical containment of the Williams report. It says that a laboratory with the highest containment level should not be established in the Netherlands, and that experiments on that level should be done at a 'European' laboratory, but it says there is a great need for facilities with containment one level lower. Regarding levels of biological containment, the commission has opted for the rules contained in the NIH regulations in the USA. For each experiment the requirements for biological containment will be left to the surveillance committee.

The commission also prefers the NIH view that certain experiments should not be carried out at the moment. The Williams report did not exclude any experiments completely. Those prohibited include the cloning of recombinant DNA from pathogenic organisms (class 3, 4 and 5) or from oncogenic viruses; the purposeful cloning of that DNA from organisms coding for the production of toxins (botulinum, diphtheriae); the establishment of a transfer of resistance against (for example) antibiotics to microorganisms missing that natural resistance; and experiments enhancing the virulence of phytopathogenic microorganisms.

On a list of examples of experiments with non-purified DNA of organisms not secreting toxic products and the conditions under which they should be carried out, the commission separates primates from other mammals, which the Williams report did not do. Concerning prokaryotes the commission distinguishes DNA from organisms which exchange genetic information with *E. coli* and those which do not. In the commission's opinion the second category carries a higher potential risk. Finally, experiments which use nucleic acid from animal viruses not pathogenic for man have to be placed in a higher category than the Williams report suggested.

So far nine projects, one of which is already in progress, have been lodged with the commission. One has to be classified as belonging to the category of research which cannot be carried out in the Netherlands; the level of two others has not been determined, while another two are P1-level experiments and two more are P2 (according to NIH regulations). Two others are P1 projects.

The establishments involved so far are the University of Amsterdam (3 projects), the Free University in Amsterdam (1), the University of Leiden (3), the University of Groningen (1) and the University of Nijmegen (1). Other universities and research institutes are preparing to do recombinant DNA research. From industry only Unilever is making preparations.

The foundation 'Biosciences and Society', which gives information on a regular basis on biomedical subjects, remarked on the report in its 31 March bulletin. It says that the commission, "consisting exclusively of prominent Dutch scientists, has hardly occupied itself with the social desirability of the recombinant DNA-research". It goes on: "The report gives no answer to the critics who doubt whether this research is socially necessary. These critics say that some results can also be reached another way." ☐

EUROPE

Wanted: a science policy

Chris Sherwell reports on a European science policy symposium held in Brussels last week

STRAITENED economic circumstances concentrate the minds of policy-makers wonderfully. The problems of those involved in science policy are worse than most, however, for the additional reason that, with science seemingly everywhere, science policy is seemingly nowhere. But with the research base an important pillar of support for the developed countries' economies, the need for science advice in government rightly remains undisputed, and the need to deal with problems with a scientific content is undiminished. So the search goes on.

As part of that search, the European Commission last week sponsored a three-day symposium at a fashionable hotel in Brussels. Ambitiously entitled "Strategies for Europe: Proposals for science and technology policies", it was organised by the private grouping known as the Science Policy Foundation, and attended by some sixty people drawn from research councils, research institutes, universities, industry and the media as well as governments.

It may remain the most important outcome of the meeting that the lines of thinking developed in recent years under the inspiration of the 'Europe + 30' idea are at least being supplemented and that concern has begun to focus on more immediate problems. If so the involvement of the Science Policy Foundation could increase, although notable absentees from the meeting, particularly from Britain's science policy establishment, may mean that any transfer of legitimacy will take time. A former Director General at the Commission, Michael Shanks, suggested in drawing conclusions from the meeting that there ought to be an organisation of science policy federations whose work could be enriched through association without being subordinate to it, and that this possibility ought to be examined, perhaps by the Commission working with the Science Policy Foundation. There was a need, he said, for the Community to have a loose network of experts independent of political allegiances who could together provide advice on common problems for leaders lacking a science education and thus help to bridge the gap between them and the broad community they served.

To this end Directorate General XII, the department of the Commission which with its responsibilities for research, science and education provided the crucial support for last week's symposium, is now engaged in preparing for two events. One is a three-day symposium which it is organising for June on the issues which the phrase "Science and Society" now commonly embraces. It is understood that this has met with resistance from some scientists and policy makers in certain member countries but has been welcomed by others. The Commission's view is guarded, but it appears prepared to see the issues given an airing under its auspices on pain of withdrawal if the experiment is as fruitless as its critics imply.

Preparations for the other event are less well finalised. It stems from the suggestion in February by Guido Brunner, the European Commissioner for both Energy and Research, of a forum or hearings on the issue of nuclear power. What form these might take remains unclear, but they are likely to be held after the proposed symposium on science and society. What is certain is that, in the aftermath of President Carter's recent announcement on nuclear power, the already strengthening worries of the Commission on the issue have been further reinforced. Faced with an increasingly threatened European nuclear programme and continuing troubles over uranium supplies from North America, the fact that Euratom and the IAEA have now agreed terms on safeguards seems to cut little ice, and this is a matter for some despair. The Carter statement confirms the difficulties of the Commission's own position: its first official response last week (the initiative will be "studied closely") was well muted.

If the symposium was anything to go by, on the science policy front it is now apparent that in Europe there is an emerging concern with two problems in particular. One is the effect of producing increasing numbers of highly qualified science graduates for whom the end of an era of university expansion has coincided with a period of inflation and recession to produce the grim spectre of unemployment. The worry is not just about where the professors of future generations will come from, or about the more fundamental matter of the state of science and research; there is also the practical worry, outside the issue of ageing research staffs, of the sheer waste involved, the opportunities foregone.

For those who choose to ignore such central matters of how much a country should devote to basic research—an issue on which researchers are more and more being called upon to defend themselves—the obvious destination for graduates is industry. But industrial innovation is the other problem over which there appears to be emerging concern in Europe: how is manufacturing industry, the so-called engine of growth, to improve its capacity to generate and harness the process and impact of innovation?

One tangible achievement of the symposium may turn out to be that it encouraged the first steps towards some amelioration of these sorts of problems. On the matter of industrial innovation, for example, in which the Commission has for some time shown an interest, for instance through its so-called Brunner - D'Avignon task force, the prospect emerged of co-operation betwen the Commission and the European Industrial Research Management Association (EIRMA), possibly with the additional assistance of the Science Policy Foundation.

Inevitably the Community's main interest, being borne of shared economic interest, is likely to focus more readily on innovation in this fashion. But it does not confine itself to that, and the symposium also confirmed that the European body that has firmly established itself as the one best placed to answer questions about the overall state of basic research throughout the continent is the Strasbourg-based European Science Foundation (ESF), made up of research councils and academies from 45 member countries.

The European Commission has close links with the ESF, and now plainly recognises its authority to speak on matters of basic research. A keynote address from Dr Gunter Schuster, Director General at DGXII, helped the ESF's President, Sir Brian Flowers, to draw a positive response later to his own intimations that the Commission could assist in efforts to establish a fellowship scheme to help build a European social science and to develop the humanities—a goal which reflects the ESF's own desire to concern itself less with the English *science* and more with the broader German *wissenschaft*. As Sir Brian said, the ESF was not concerned with studying policy; by its activity it contributed to it.

For most practising researchers that view will probably provide some real comfort at a time when their need is not so much for science policy studies as for science policy itself. The question remains whether they can represent this interest successfully. ☐

WEST GERMANY

The cost of maintenance

Werner Gries reports from Bonn on West Germany's funding for science

WEST GERMANY spent DM24,200 million on scientific research and development in 1976: DM12,700 million came from the government, DM10,900 million from industry and the remainder from overseas and from independent foundations. As is customary, 55% of the government contribution was raised by the central government, and 45% by the *Länder* which comprise the federal republic. In contrast to other Western nations, military research and development plays a comparatively minor role in West Germany: in 1976 it received DM1,500 million—6% of the total research expenditure. Since 1973 there has been no increase in the resources available for research and development and plans for 1977 show that in real terms, total expenditure on research will decrease. In 1976 2.2% of GNP was spent on scientific research.

Non-nuclear energy research is receiving increasing support from both the government and industry. The government contribution for specific projects connected with non-nuclear energy amounts to DM270 million in 1977. Some DM300 million will be spent on research centres and other projects. Mining and coal technology are the most important areas receiving government subsidy. New techniques of searching for and developing petroleum, natural gas and uranium are also being investigated, as are the possibilities of solar and wind energy. Solar collectors for heating are now so advanced that the government is considering offering special incentives, particularly investment bonuses, to encourage manfacturers to put new techniques on the market. The government is also subsidising research into new methods of saving energy.

On the nuclear front, West Germany is, amongst other things, developing the fast breeder reactor. About 2,300 people are now employed on the development work and in 1977 about DM380 million will be made available to research establishments and industry for further development. The principal project is the 300 MW prototype reactor at Kalkar, which according to the latest estimates will cost DM2,800 million to set up. A smaller experimental reactor is being built in Karlsruhe to test the fuel elements for the fast breeder. Plans are also going ahead for the construction of larger fast breeder reactors in collaboration with France. German electricity supply companies are involved in the construction of the French Super-Phénix, for which they are receiving a grant of DM55 million from the West German government.

The Ministry of Research and Technology is in charge of all government activities in the field of fast breeder development, and although there is growing opposition to these reactors in parliament, the Ministry apparently intends to continue promoting intensive research on them. So far the government alone has spent DM2,100 million on the development of fast breeder reactors. In addition to the prototype at Kalkar, a large fast breeder reactor is scheduled to be built in the early 1980s. Its construction will take into account the results of the French project; there has been close cooperation between France and West Germany on fast breeder development since the treaty between the two countries in 1975.

In 1977 space research will receive DM600 million almost exclusively from the government, and in particular from the Ministry of Research and Technology: DM347 million will go to the European Space Agency and form the major part of West Germany's spending on space research. At the moment the West German government is primarily involved in financing the ESA Spacelab and in the development of various exploratory satellites. It gives DM 40 million a year towards developing the launcher Ariane; an extra DM 10 million will go towards the building of a launching base for the Ariane in Kourou in French Guiana.

Support for national space projects has decreased steadily in the past few years and now only 20% of the total expenditure goes on domestic projects. In 1977 DM133 million will be spent on a large research establishment for air and space travel, Deutsche Forschung-und Versuchsanstalt für Luft und Raumfahrt (DFVLR). ☐

SWEDEN

A catalytic note

Wendy Barnaby reports from Stockholm on Sweden's recently established Energy Institute

SWEDEN'S new international Energy Institute, opened recently in Stockholm, is planning to carve a niche for itself in research on energy/ecology problems. It will see man's use of energy as a special case of the energy flow in the biosphere, affecting it and all natural resources. This should be a suitable focus for the new Director, Professor Gordon Goodman, a British ecologist with wide experience in energy research, and it is in line with the interests of the chairman of the international board, Professor Jack Hollander, an American who heads a large project on energy for the US Academy of Science.

Specific research projects will depend largely on the results of the review of current energy research that Goodman and a plasma physicist, Lars Kristoferson, are about to begin—both want energy researchers to notify them of projects being done. The idea is to close those gaps created by the transdisciplinary nature, long time-scale or low economic interest of the work needed to fill them. The Institute will not therefore be interested in short-term, controversial topics; Goodman wants to establish its reputation as a trusted supplier of timely and significant facts for the international debate on energy-related problems. He envisages the Institute playing a catalytic role by preparing review papers highlighting the need for research in various areas. As it has no laboratory facilities, its work will be carried on by seminars, symposia and publications.

The Institute's funds are very limited—about $400,000 a year for the next seven years from the private Beijer Foundation—and additional funds will have to be sought elsewhere to get a reasonable programme going. But Goodman points out that the money has no strings attached and will enable the Institute to avoid projects meant to bolster sectarian interests. Because of the financial limitations, permanent positions will probably be limited to the Director and his assistant; academics coming to Stockholm for short visits or sabbaticals would help carry out projects. ☐

IN BRIEF

JET meeting

The Committee of Permanent Representatives (COREPER) in Brussels, made up of senior officials of the EEC member states, meets this week to discuss the ill-starred joint fusion project, JET. If they can resolve the outstanding problem of the method by which the proposed JET council will work, the way will be clear for an early Council of Ministers meeting to finalise the question of the site, which is expected to be either Garching in Germany or Culham in England.

Argentinian disappears

The latest Argentinian scientist about whom concern is being expressed is Horacio Dottori, an astrophysicist. Sr Dottori was at the Cordoba Astronomical Observatory from 1967 to 1976 except for a two-year period in Germany at the Max Planck Institut für Physik und Astrophysik from 1971–73. He worked on observations of peculiar and interacting galaxies. In March 1976, following the military takeover, he was dismissed. He was arrested in August 1976 and has now disappeared; his friends believe he has been sent to a camp called 'La Perla', about which little is known.

X-ray survey

To meet the requirements of a directive on radiological protection from the EEC Council of Ministers, and because existing information in Britain is out of date, the UK National Radiological Protection Board (NRPB) is to conduct a survey, beginning in June and encompassing over 100 National Health Service hospitals, of the radiation doses which patients receive from the diagnostic use of X-rays.

The medical use of radiation contributes some 13% of the total dose which the population receives, making it the largest man-made component. Natural background radiation contributes some 84%. The specific objective of the survey is an estimation of the so-called 'genetically significant dose' (GSD) to the population arising from diagnostic radiology.

Chemistry PhD survey

The Chemical Society's Standing Advisory Committee on Relationships between Higher Education and Industry (SACRHEI) recently completed a survey on US views of the British chemistry PhD. It sought the opinions of 40 US industrialists, seven US professors of chemistry and a couple of professional associations. Although about half the industrialists and academics thought the British and American PhDs comparable so far as research is concerned, the industrialists thought holders of British chemistry PhDs were less motivated towards employment in industry and had less appreciation of overall industrial problems than their American counterparts.

Consumer revolt

THOMAS H. JUKES

THE ban on saccharin proposed by the Food and Drug Administration (FDA) has unleashed a public reaction of unexpected, and allegedly unprecedented, intensity. The protest against the ban was apparently expected to last for only a few days and, accordingly, the US manufacturer of saccharin announced plans to discontinue production. After a week, these plans were cancelled because of the heavy and continuing demand for saccharin by consumers who were stockpiling it in various forms. This must have been a great surprise to those who thought that mere mention of carcinogenesis would make the public abjure saccharin.

I have received copies of about 150 US newspaper articles on the ban. The FDA came in for a tremendous clobbering, even though its action was mandatory under provisions of the Delaney Clause of the Federal Food Drug and Cosmetic Act. In former days, those who objected to the Clause were often rebutted by *ad hominem* accusations, as for example by Epstein (*Preventive Medicine* **2**, 140, 1973):

> All such criticisms [of the Delaney Clause] have emerged from industrial groups, trade associations and their consultants, and from groups that are clearly identified with protection of industrial interest . . . Such 'captive' spokesmen [are characterised by] lack of expertise and national recognition in the field of chemical carcinogenesis . . . No such criticisms have emerged . . . from qualified scientific representatives of citizen, consumer and public interest groups.

In the face of such invective, a symposium on the Delaney Clause, held by the New York Academy of Sciences in 1973, did not produce a single proposal for its revision. Banning saccharin seems to have changed the picture. As a columnist said, "It wasn't large corporation people who swept through supermarkets, cleaning the shelves of artificially sweetened products".

The FDA, perhaps intentionally, noted that "a human would have to drink 800 12-ounce cans of diet soda a day for a lifetime" to ingest an amount of saccharin comparable to that received by rats in the Canadian study that led to the ban. In vain did FDA point out that one can daily would still impose a proportionately lesser risk. Cartoonists, columnists, and lawmakers swooped down gleefully on the 800-can statistic. One cartoon showed a pharmacist offering a frustrated saccharin customer cigarettes and whisky as an alternative for the banned sweetener. A congressman sought fleeting fame by introducing a bill to prohibit giving saccharin to rats. "Science For The People" had taken over, but not in the way that its sponsors had originally planned. A few stern hard-liners told diabetics to stop griping, and drink water, a sort of Marie-Antoinette-in-reverse suggestion that was not well received.

Public rebuff of the Delaney Clause received support from eminent clinicians who said they had seen practically no cases of bladder cancer in diabetics who had been on saccharin for many years, thus implying that rats responded differently from human beings. Thus the two cardinal points of the Delaney Clause: extrapolation of carcinogenesis from laboratory animals to human beings, and the absence of a threshold, both were widely discounted. The Delaney Clause may well need revisions. However, let us hope that opposition to banning saccharin will not jeopardise a programme to reduce exposure to environmental carcinogens.

As of last reports, the FDA was still answering telephone calls, but Congressman James Delaney could not be reached.

correspondence

Rights of scientists

SIR,—You quote the newly published report *Scholarly Freedom and Human Rights* (24 February, page 671) in stressing the responsibility of learned societies to support oppressed and persecuted scientists, by public protests. This is important: hitherto it has usually been argued that only non-scientific channels are permissible for such protests because actions taken by scientific societies would be political demonstrations not consistent with their high scientific aims.

Two points seem to be disregarded in this argument. Firstly, that protests through non-scientific channels emphasise the humanitarian aspect whereas the professional aspect is emphasised only through scientific societies. If a scientist is hindered or restricted in his work and prevented from normal communication and exchange with his colleagues within the international scientific community, not only is his own work impeded but also that of his colleagues and hence scientific progress in general. Secondly, that the governments that persecute scientists bring politics into science whereas the scientific societies submit their professional protests just to keep politics out of science.

It has also been argued that international societies should be particularly careful in such matters, whereas national societies might act more freely. However, there are good reasons for the view that an international scientific society is an appropriate channel for appeals and protests when scientists are arbitrarily cut off from the international forum of scientific discussion. The very purpose of an international scientific society is to promote science by fostering international collaboration, communication and exchange among scientists. A national society is involved in professional, educational and advisory activities, and the promotion of international cooperation, which is the main concern of an international society, is of but minor importance. If a member of an international society is prevented from normal communication and exchange with fellow-scientists in other countries, the very purpose of the society is violated and it is the duty of its board to take measures to restore the member to a normal scientific life. Although practical considerations may call for restraint and moderation while acting within an international body, it is important that the principle is made clear. It is the scientific and professional aspect of the matter that should be advanced in this context; political and other arguments should be avoided.

In conclusion, then, it is the duty of scientific societies to speak up officially in defence of oppressed and persecuted scientists.

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The Tacoma Bridge collapse

SIR,—Dr Henry Chilver (10 February, page 494) reviewed a number of accidents and attempted to categorise them by making use of a 'triangle of failures'. The failure of the Tacoma Bridge in 1940 lies in Chilver's diagram midway between 'unknown environmental forces' and 'unpredictable behaviour' and he says that "The cause of the accident was an inadequate understanding of the interaction of the structure with its environment, leading to large oscillations of the bridge. The resulting behaviour was unpredictable at the time . . . ". There were however indications in information available at that time, but not collated then, that a failure would occur and that the mode of failure was not new. The figure shows variation of span length, L, of a suspension bridge, with the year of building. Also shown are corresponding values of the ratio of girder depth, d, to span length (some examples are missing because records are incomplete); this parameter is a rough measure of the stiffness of the girder against vertical loads from wind or traffic. In the 19th century, bridges were designed on an intuitive basis and several were destroyed or damaged by winds. In one or two instances, eye witness reports were recorded and these bear remarkable similarity to the observations recorded on film during the collapse at Tacoma. The earlier collapses consistently occurred whenever d/L was less than approximately 0.006.

At the turn of the century a new theory was developed which seemingly gave mathematical rigour to the design of the deck girder. This theory considered only static wind pressure loading but, for the heavy, deep girders then currently fashionable, it proved very successful and no dynamic behaviour was observed. During the next thirty years several suspension bridges were built and, with the confidence instilled in the designers by the availability of the theory, there was a trend to much lighter girders (d/L). Economy, lower traffic loads and aesthetic appeal were the main driving forces in this move. Eventually, as the figure shows, the values of d/L dropped below 0.006 and wind induced oscillations were observed in a number of bridges, although only the Tacoma Bridge actually collapsed. After that deck girders were made stiffer, but the pattern of development was quickly re-established. (This time, however, the critical value of d/L has been safely reduced because of the use of wind-tunnel testing in conjunction with the design calculations).

Had proper records been kept of trends in bridge design, and had engineers been made aware of earlier wind-induced failures, a warning would have been sounded on the dangers of using the analysis for very light deck girders. We have shown that the failures of other bridges of different types could also have been avoided if records had been kept of design trends and minor accidents preceding the major event had been adequately investigated. Similar trends have been noted in structures other than bridges. We suggest the need for a facility to gather and process information on which to base the direction of research on structural behaviour.

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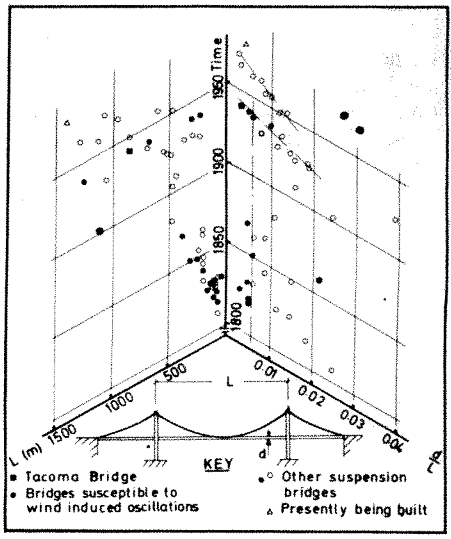


news and views

Another optical pulsar

from Jocelyn Bell Burnell

EVER since the discovery of optical flashes from the pulsar in the Crab Nebula and the association of pulsation in this band with rapid pulsation period, astronomers have expected that the Vela pulsar, the third fastest, should likewise emit pulses of light. But in the 8 years since that initial discovery hope has gradually faded as larger and larger telescopes have searched for fainter and fainter flashes. So far had faith dimmed that consideration was recently given to the unpulsed optical emission of the pulsar, and an attempt made to pick out the candidate star on the basis of predicted magnitude and colour (Lasker *Astrophys. J.* **203**, 193; 1976).

But at last, optical pulsations have been found. In this issue of *Nature* (page 692) Wallace and eleven others (in a paper with more authors than paragraphs) report the detection of optical pulsations from the Vela pulsar. In a collaborative effort between optical astronomers from the Royal Greenwich Observatory and the Anglo-Australian Telescope, and radio astronomers in Australia, faint optical pulses at the same pulsation period as the radio pulses have been recorded. With a time-averaged magnitude of 25.2 this is the faintest thing ever seen (and in good agreement with Pacini's prediction that it would have magnitude about 25 (*Astrophys. J.* **163**, L17; 1971)).

The Vela pulsar has been a particularly troublesome pulsar to deal with. Not only are the light pulsations weak, requiring a large modern telescope such as the AAT with sophisticated equipment for analysis, but the pulsation period of the pulsar itself, as observed by the radio astronomers, is not stable. Pulsars are believed to be rotating neutron stars—stars of a few kilometres radius with a density comparable to that found in the atomic nucleus. The pulsation period is the rotation period, which in the case of the Vela pulsar is 80 ms. The period gradually lengthens as the star loses rotational energy, and, again for the Vela pulsar, this is about 11 ns per day. This is typical pulsar behaviour, although for both the Crab and Vela pulsars the rotation rates are much faster than average and the slowing more noticeable. What is peculiar to the Vela pulsar and partly responsible for the delay in the discovery of the light flashes is the occasional large hiccough or "glitch" of the pulsar, when its rotation rate suddenly increases (typically by 200 ns). The position of a radio pulsar is obtained by the radio astronomers from a study of the Doppler shifts in its pulsation period due to the annual motion of the Earth about the Sun. Erratic behaviour of the pulsar period upsets the determination and uncertainties in the position of this pulsar have not helped the optical astronomers.

The region around the Vela pulsar, PSR 0833-45, is complex. There are many faint stars, and the remains of a supernova—a star that exploded cataclysmically blowing off a shell of material. The pulsar, which may have been formed by the supernova explosion, lies within part of this shell—the part called Vela X by radio astronomers. To add confusion to complexity there are two X-ray sources in the constellation of Vela, Vela X-1 (3U0900-40) and Vela XR-1 (3U0833-45). Vela X-1 shows strong X-ray pulsations whereas Vela XR-1 which is presumably associated with the pulsar does not!

According to Pacini's predictions the pulsated X-ray emission from 0833-45 should be below the threshold of present X-ray telescopes, but γ-ray astronomers have had noticeable success in detecting this pulsar. (The division between X and γ-ray astronomy is made on the basis of photon energy, the divide being around a few hundred keV, and is independent of the method of production of the photons.) So successful have been the γ-ray astronomers (with the COS B satellite, for example), that one wonders whether it might not be reasonably strong in X rays too. It will be interesting to learn more about the X-ray source: where it is in relation to the pulsar, what it is, and what fraction of the X-ray emission is pulsed.

It is tempting to assume that the Vela pulsar is similar to the Crab only less energetic: both are rapid pulsars, both emit pulses at the radio pulse period over a wide range of the electromagnetic spectrum and at γ-ray energies their pulse profiles are strikingly similar. But there are striking differences too, notably that whereas for the Crab the radio, optical, X- and γ-ray pulses are all in phase, in the Vela pulsar the radio pulse apparently precedes the optical and γ-ray pulse.

One is reluctant to divide a sample of two into two separate categories, and, unfortunately, there are unlikely to be many more samples on which to establish such a division. This makes this discovery and the consequent observations all the more important. ☐

Correction

Changes in proof to the article by Roberts and Falkow in last week's *Nature* (**266**, 630; 1977) require the following correction to be made to the accompanying comment in *News and Views* (**266**, 586; 1977). In paragraph 3 it is stated that the 25 Mdal sex-factor plasmid has not yet been found to be transferred to recipient strains along with the drug resistance plasmid. Roberts and Falkow have now found that it is transferred to recipient strains of *Neisseria gonorrhoeae*.

Synaptic memory in the hippocampus

from Shin-Ho Chung

IT has been known for some time that efficiency in synaptic transmission can be enhanced for several hours after presynaptic fibres have been briefly stimulated. Interest in this phenomenon, known as 'long-lasting potentiation', is partly due to the belief that such a change in synaptic efficiency may well be a neurophysiological mechanism for storage of information in the central nervous system. While most physiological studies on synapses have emphasised events occurring immediately after the arrival of a nerve impulse, psychological observations reveal many phenomena which must be due to long-lasting effects of nerve impulses on synaptic pathways. Amongst these are the after-effects of intense sensory stimulation, which may persist for many days, as well as conditioned reflexes, and the process of learning; all these are almost certainly due to long-lasting changes in the properties of neurones and synapses brought about by use and disuse. A number of recent studies are aimed at elucidating the underlying mechanisms of synaptic plasticity.

Long-lasting potentiation in a mammalian synaptic pathway was first discovered by Larrabee and Bronk (*J. Neurophysiol.* **10**, 139; 1947) and was later confirmed by Lloyd (*J. gen. Physiol.* **33**, 147; 1949). In a simple synaptic pathway where the presynaptic axons connect directly to the postsynaptic neurones, they noted that transmission through the pathway is greatly enhanced for several minutes following a brief period of repetitive stimulation of the presynaptic axons. The duration of this facilitation could be extended for up to 2 hours by stimulating the pathway at an optimal frequency (Beswick & Conroy *J. Physiol. Lond.* **180**, 134; 1965). Bliss and Lømo (*J. Physiol. Lond.* **232**, 331; 1973) later extended this observation to the hippocampus, which proved to be a particularly suitable structure for electrophysiological analysis of synaptic plasticity. Cells there form tightly packed layers, with their dendrites oriented radially, and afferent inputs originating from different regions of the brain terminate at discrete segments of the dendrites (see diagram on page 738 of this issue of *Nature*). Brief episodes of repetitive stimulation to the perforant path fibres, which link the entorhinal cortex with the granule cells of the hippocampus, produce a cumulative increase in the amplitude of the responses evoked by subsequent single stimuli. Compared with the spinal cord (Lloyd *op. cit.*; Beswick & Conroy *op. cit.*), the duration of synaptic potentiation in the hippocampus is prolonged, and the degree of facilitation pronounced. For example, a few brief trains of repetitive stimulation increase the amplitude of the synaptic potential by as much as 300% and this enhanced synaptic efficiency persists, in certain conditions, as long as several weeks (Bliss & Gardner-Medwin *J. Physiol. Lond.* **232**, 357; 1973). Since these initial reports, the phenomenon has been confirmed in various mammalian species and in two other hippocampal pathways (Deadwyler *et al. Brain Res.* **88**, 80; 1975; Douglas & Goddard *Brain Res.* **86**, 205; 1975; Schwartzkroin & Wester *Brain Res.* **89**, 107; 1975; Dudek *et al. J. Neurophysiol.* **39**, 384; 1976).

An unresolved question, which Anderson *et al.* (page 736 of this issue of *Nature*) and Lynch *et al.* (page 737) have sought to answer, is whether the enhanced synaptic efficiency seen in the conditioned pathway is due to a generalised increase of sensitivity in the postsynaptic neurones, or whether it is confined to those synapses which have been repetitively activated (or tetanised). Although the findings reported by the two groups differ in detail, it is now satisfactorily established that a prolonged increase in synaptic efficiency is confined to the tetanised pathway, suggesting that the underlying processes giving rise to the hippocampal potentiation may differ from those responsible for the heterosynaptic facilitation which occurs in certain invertebrate neurones (Kandel & Tauc *Nature* **202**, 145; 1964; *J. Physiol. Lond.* **181**, 1; 1965). Andersen and his colleagues report that a tetanus applied to one group of afferents causes no change in synaptic transmission in the untetanised pathway, while Lynch and his coworkers claim that the enhanced synaptic efficiency in the tetanised pathway is accompanied by a generalised depression of the postsynaptic neurones. This latter result is consistent with their previous finding that the sensitivity of pyramidal cells to iontophoretically applied glutamic acid is decreased after tetanus (Lynch *et al. Nature* **263**, 151; 1976). While the conflicting observations reported by the two groups are difficult to reconcile, they are only tangential to the ultimate aim of the investigation, which is to localise the changes in the properties of neurones and synapses caused by use and disuse, and to understand the ways in which these changes are induced.

There are a number of possible mechanisms which may be responsible for long-lasting potentiation (see Bliss & Lømo *op. cit.*). For example, an impulse flowing down the previously tetanised pathway may invade more terminals than normal, thereby producing a larger effect on its target cells (see *News and Views, Nature,* **264**, 313; 1976). Alternatively, and this is the possibility which Andersen *et al.* favour, the same number of terminals may be activated but each terminal may liberate an increased amount of transmitter. Finally, it is also possible that previous activity may increase the sensitivity of the postsynaptic junctional membrane; for example, Rall (*Biophys. J.* **2**, 145; 1962), among others, has postulated that a structural alteration of the postsynaptic spines may be an important variable in changing the relative weights of synaptic inputs. The swelling of the spines, due to the uptake of electrolytes and water, can cause a reduction in the resistance of the narrow stems by which the spines are attached to the parent dendrites, and this could provide a basic mechanism for long-lasting potentiation in this monosynaptic pathway. In this context, it is relevant to note that the diameter of spines in the hippocampus appears to increase abruptly when the pathway innervating them is tetanised (van Harreveld & Fifkova *Anat. Rec.* **181**, 355; 1975; *Expl Neurol.* **49**, 736; 1975).

The molecular basis for long-lasting potentiation will remain speculative until synaptic transmitters in the central nervous system are identified, and this is proving an elusive task. An excellent study in the amphibian sympathetic ganglion, where the synapses are known to be mediated by acetylcholine, has demonstrated that a slow decrease in membrane conductance, triggered by muscarinic receptors, causes potentiation of synaptic responses generated by the activation of nicotinic receptors (Schulman & Weight *Science* **194**, 1437; 1976). Athough the mechanism of potentiation in the hippocampal pathways may not be the same as in the amphibian sympathetic ganglion, it is this type of controlled study which is now needed for cortical cells. Once the underlying mechanism governing plasticity observed in the simplest synaptic system is elucidated, experimental investigation can then be extended to the more complex polysynaptic pathways and, finally, to the grey matter of the cerebral cortex. This ultimate aim, however, will not be achieved in the near future. As Ramón y Cajal once remarked, the structure of the cerebral grey matter is so intricate that it defies and will continue to defy for many decades the obstinate curiosity of neurobiologists. □

Anti-anxiety receptors in the brain?

from Leslie Iversen

LIGAND binding techniques are well established as important tools for studies of a variety of biological receptors for peptide hormones and neurotransmitter substances. In most cases such assays have been developed to investigate receptors for which the naturally occurring ligand is already known, for example, insulin, acetylcholine or noradrenaline. In the case of the opiate receptor, however, the discovery of the high affinity binding of radioactively labelled morphine-like drugs to membranes in brain homogenates (Pert & Snyder *Science*, **179,** 1011; 1973) was of a different character, since it allowed description of the precise characteristics and distribution of a receptor mechanism for which no naturally occurring ligand was then known, and it offered a powerful tool for the subsequent discovery of the enkephalins and endorphins as a new class of naturally occurring agonists for a receptor mechanism hitherto described only in terms of the organism's response to opiate drugs.

Such drugs, however, represent only one of several important classes of centrally acting drugs whose pharmacological mechanisms remain obscure. Squires and Braestrup (page 732 of this issue of *Nature*) now describe their preliminary results in applying a similar rationale to studies of the receptor mechanisms possibly involved in mediating the actions of minor tranquillisers of the benzodiazepine class. These drugs, exemplified by the anti-anxiety agent diazepam (Valium) and the hypnotic drug nitrazepam (Mogadon), have had an enormous commercial success since the discovery of their remarkable psychopharmacological properties in the 1950s. They, and many related analogues in the benzodiazepine series, are able to tame otherwise aggressive laboratory animals, they exhibit CNS muscle relaxant properties, and in man the drugs are used as anticonvulsants and more importantly as anti-anxiety agents. At higher doses they are also effective hypnotics, and since they are much less toxic than barbiturates, have taken an increasing share of the large market for those suffering from anxiety and/or insomnia. Benzodiazepines, needless to say, have been consumed by many millions of people in the industralised nations of the world in which such complaints are endemic.

Despite their unique pharmacological properties and widespread medical use, the mode of action of the benzodiazepines remains unknown. There have been various attempts to explain their effects in terms of current neuropharmacological fashion, that is, by action on CNS catecholamine or indolamine transmitters, but these have been largely unconvincing. Young *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **71,** 2246; 1974) found that the potencies of a series of benzodiazepines in displacing radioactively labelled strychnine from binding sites associated with glycine receptors in mammalian spinal cord correlated fairly well with the potencies of the same drugs in various animal behaviour tests predictive of anti-anxiety properties, and these authors proposed the hypothesis that the drugs might interact with receptors for this inhibitory amino acid transmitter in CNS. However, relatively high concentrations of the drugs were needed to show this effect and subsequent neurophysiological experiments failed to lend support to this hypothesis (Curtis *et al. Brit. J. Pharmac.* **56,** 307; 1976). A more recent proposal has been that the benzodiazepines might in some way mimic or enhance the effects of another inhibitory amino acid transmitter, γ-aminobutyric acid (GABA) at CNS receptor sites. This hypothesis was based on both biochemical and neurophysiological evidence (Polc *et al. N. S. Archiv. Pharmac.* **284,** 319; 1974; Costa *et al. Adv. Biochem. Psychopharmac.* **14,** 113; 1975), and has attracted a wide following. Nevertheless, it has proved difficult to demonstrate any direct interaction between benzodiazepines and GABA receptors, although recent neurophysiological evidence has shown that these drugs are able to exert inhibitory effects on the firing of single cells in rat brain similar to those seen on application of GABA (Dray & Straughan *J. Pharm. Pharmac.* **28,** 314; 1976).

Squires and Braestrup found that high specific activity ^{3}H-diazepam is bound to saturable sites in rat brain membrane preparations with very high affinity—half saturation being achieved at a drug concentration of only 2.6 nM. When low concentrations of the labelled drug were used more than 90% of the total binding was attributable to this single class of high affinity sites. More importantly, they showed that the potencies of 21 different non-radioactive benzodiazepine drugs in competing with labelled diazepam for binding showed a highly significant correlation with the known pharmacological potencies of these drugs determined previously in various animal tests known to be predictive for anti-anxiety properties in man. The potencies of the drugs tested in both the *in vivo* and *in vitro* tests ranged over four orders of magnitude. On the other hand, none of the known amine or amino acid transmitters (including glycine and GABA), or any of the drugs known to interact with their receptors, had any inhibitory effects on ^{3}H-diazepam binding. Other minor tranquillisers, such as meprobamate, barbiturates or ethanol, were also ineffective as inhibitors, as were opiate drugs.

The authors therefore, claim to have described a saturable high affinity binding mechanism whose pharmacological specificity suggests that it may have some important relation to the mode of action of the benzodiazepines in whole animal experiments or in man. It is also interesting to note that the highest density of ^{3}H-diazepam binding sites was found in the frontal and occipital cortex of rat brain, areas not known to be enriched in any of the known transmitter substances.

If their hypothesis is correct, and caution is certainly still needed in extrapolating from these preliminary biochemical results, their findings may have a major impact on our future understanding of the mode of action of this class of psychotropic drugs. By analogy with the discovery of the opiate receptor, the benzodiazepine receptor assay may even offer a simple means for the discovery of the naturally occurring ligand for such receptors—the brain's own anti-anxiety substance? Further developments in this field will certainly be awaited—anxiously.

A hundred years ago

AT the last meeting of the French Anthropological Society, a long report was read which showed that Druidism was not quite extinct in Britanny, some country people still adhering to Pagan practices in spite of the priests' exertions. It was noticed that the clergy were anxious to destroy menhirs and other similar relics. A petition has been sent to the ministry to put a stop to this iconoclastic zeal.
From *Nature*, **15** 19 April, 539; 1877.

SIN and the muon-electron puzzle

from F. E. Close

DURING the past few weeks several papers have appeared proposing mechanisms to explain the exotic radiative transmutation of the muon into the electron. Although widespread publicity in the world press may have led readers to think that this 'forbidden' decay has been seen (hence the sudden flux of theoretical papers) no one involved in the experiment at the Swiss Institute for Nuclear Research (SIN) in Zurich has yet claimed to have solid evidence for it. Indeed, the group concerned are still quoting a limit consistent with no such decays at a level of about one part in a hundred million of all muon decays. The rumours, and source of the press reports, were that a signal was being seen at the level of order one part in 10^9. However, there are experimental questions still to be settled, for example, the energy and angular resolutions which limit conclusions at present: hence the present claim only of a limit.

Why all the fuss? The reason is that for over a quarter of a century the muon has remained a mystery. So far as we know the Universe could have existed without a muon at all, and our everyday experiences in such a Universe would have been the same as in the real world (apart from esoteric phenomena like the pion lifetime). Yet for some reason nature is not satisfied with the electron and its associated neutrino and has repeated itself by invoking two more leptons (the muon and its neutrino).

The muon is identical to the electron in all respects except that it is about 200 times as massive. The question 'why does the muon weigh?' has remained unanswered for 30 years. No evidence for a new interaction possessed by the muon has been found, which would explain its larger mass and distinguish it from the electron. In all respects the muon appears to be simply a heavy electron and yet does not decay radiatively into one. It seems that the muon remembers that it is a muon partnered with its own neutrino and so

$$\mu^- \rightarrow \nu_\mu + (e^- + \bar{\nu}_e)$$

occurs but decay modes like $\mu^- \rightarrow e^-\gamma$, $e^-e^+e^-$ and so on are not seen. A rule was therefore invented to dignify these observations: muon number (possessed only by the muon and its neutrino) is conserved in nature. Beyond this little or no progress was made.

Key

γ,	photon
Leptons	
e,	electron
e^+,	positron
ν_e,	electron neutrino
$\bar{\nu}_e$,	electron antineutrino
μ,	muon
μ_e,	muon neutrino
$\bar{\mu}_e$,	muon antineutrino
Hadrons	
p,	proton
Λ,	lambda
π,	pion
K,	kaon
D°,	neutral charmed meson

Early searches for the radiative decay $\mu \rightarrow e\gamma$ were made and Pontecorvo and Hincks found a limit of a few per cent of all decays. Over the years this limit has been greatly improved and today stands at the level of one part in a hundred million. In principle experiments currently in progress at SIN in Zurich and at TRIUMF in Canada will either see a signal or place new limits of order one part in a thousand million. There are reports of an experiment planned at Los Alamos, New Mexico, which may be able to go down to a sensitivity of 1 part in 10^{10}.

The dramatic significance of these limits may be appreciated when one realises that only the known muon decay mode $\mu \rightarrow e\nu\bar{\nu}$ is a typical weak interaction. Hence one is failing to see $\mu \rightarrow e\gamma$ (naively an electromagnetic transition) at less than one hundred millionth of a weak decay rate (this latter being the weakest interaction apart from gravity that is known to exist). Hence, if there are any undreamt of subtle effects in the weak interactions then it may well turn out that they will show up first in the $\mu \rightarrow e\gamma$ amplitude.

Gauge theories and massive neutrinos The discoveries of charmed particles and their intimate relation with the gauge theories have painted a clear and beautiful picture of nature. An intimate deep relation has been noticed between the lepton world and the quark world, more specifically the ($\mu\nu_\mu$) pair *vis à vis* the ($e\nu_e$) pair in the leptons on the one hand and the charmed–strange quark pair *vis à vis* the up–down quarks in the hadron world on the other. In the future this may point towards a unification of leptons and quarks (or weak–electromagnetic and strong interactions!). At present it has made theorists think again about the role of the muon in nature's scheme—why it exists and why it does not decay radiatively into the electron. To appreciate these ideas it is useful first to describe the salient points of the current theory and then describe the new ideas on the application to the muon and its decays.

The carriers of the electromagnetic and weak interactions are the photon and the W boson respectively. When these fields interact with leptons or with quarks they can cause transmutations only within the left-handed doublets of

$$\text{leptons: } \begin{bmatrix} \nu_e \\ e \end{bmatrix} \begin{bmatrix} \nu_\mu \\ \mu \end{bmatrix} \text{ and quarks: } \begin{bmatrix} u \\ d' \end{bmatrix} \begin{bmatrix} c \\ s' \end{bmatrix}$$

Quarks d, s with well defined mass (eigenstates of the strong interactions) are linear combinations of the d′ and s′

$$d' = d\cos\theta + s\sin\theta$$
$$s' = -d\sin\theta + s\cos\theta.$$

It is as if the strong interaction world is rotated by a small angle θ (the Cabibbo angle) relative to that of the weak interactions. Hence the weak interactions can, and do, cause transitions between the (ud) quark doublet and the (cs) doublet. It is by this 'leakage' that the Λ beta decay, $\Lambda \rightarrow pe\bar{\nu}_e$ occurs and by which the charmed particles decay

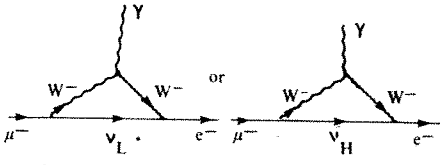
$$\begin{cases} c \rightarrow s' \equiv s\cos\theta - d\sin\theta \\ D^\circ(c\bar{u}) \rightarrow K^-(s\bar{u})\pi^+ \end{cases}$$

This mechanism for charmed decay is known as the GIM mechanism (see *Nature* **262,** 537; 1976) due to S. Glashow, J. Iliopoulos and L. Maiani. Is there an analogous mixing and GIM mechanism for the lepton doublets? In principle the answer is yes, and a consequence is that a leakage from muon world to electron world can arise leading in particular to the radiative decay $\mu \rightarrow e\gamma$. How does this come about?

Suppose that the two neutrinos have different masses. Then we can define ν_L and ν_H for the lighter and heavier respectively and these will play the part of the d and s quarks. The partners of the electron and muon in weak interactions will be defined as ν_e and ν_μ (these playing the part of the d′ and s′ quarks). Then analogous to the quark system the $\nu_{L,H}$ may be orthogonal mixtures of ν_e, ν_μ.

$$\nu_e = \nu_L\cos\ + \nu_H\sin\varphi$$
$$\nu_\mu = -\nu_L\sin\varphi + \nu_H\cos$$

the decay $\mu \rightarrow e\gamma$ can then proceed for instance by the processes



If the L and H neutrinos have the same mass (in particular being both massless) then there is exact cancellation between the two and the decay mode vanishes. However, if there is a mass difference then the amplitude is finally proportional to $m_H - m_L$ scaled by m_W, the mass of the W boson.

The W boson is believed to have a mass of around 60 GeV. The mass difference of the two neutrinos is at most some 650 keV (it could even be zero) and so the predicted rate can at most be

$$(\mu \to e\gamma)/(\mu \to \text{all}) \lesssim 10^{-25}$$

which is far below the present limits.

New heavy leptons

In the light of the above, what price must we pay if the decay were found at a level of 1 in 10^9 or so? This could have been accommodated in the above arguments if the heavy neutrino has a mass of several GeV, far in excess of the known limit. Could there be further heavy leptons in nature which could help in this respect? There is now good evidence for a heavy charged lepton τ around 2 GeV in mass, probably accompanied by a neutral ν_τ. The latter has a mass less than 700 MeV (Perl *Phys. Lett.* **63B**, 466) and so cannot be of use in the present context. However, this discovery does release one's inhibitions and shows that a whole new spectroscopy of heavy leptons is not necessarily absurd.

If such further heavy leptons indeed exist then the $\mu \to e\gamma$ decay can arise and several mechanisms have been suggested. One way is to have right handed doublets involving new neutrals and the electron or muon. With the massive neutrals introduced this way the previous game can be played and a sizeable $\mu \to e\gamma$ decay accommodated. This idea has been proposed by T. Cheng and L. Li in the USA. An interesting by-product could arise in connection with the failure to see parity violation in atoms. Parity violation occurs if the electron (muon) only takes part in left handed weak interactions. If the electron (muon) now also couple to heavy neutral leptons in the right handed mode, parity can be conserved. (Of course, parity will still be violated in neutron β decay as the neutron–proton mass difference is so small that only the electron and its massless left-handed neutrino can be produced). In a similar vein, F. Wilczek and A. Zee of Princeton University have proposed doubly charged heavy leptons to generate the μ decay.

Before we conclude that the observation of $\mu \to e\gamma$ would force us to deduce that heavy leptons exist we should notice a proposal of J. Bjorken and S. Weinberg of Stanford, California. In the gauge theories, particles gain masses by coupling to scalar fields known as Higgs particles. The coupling of the Higgs particles to fermions can generate contributions to weak interaction decay rates. However this is usually ignored since the coupling is proportional in strength to the masses of the fermions concerned, and the electron and muon are very light. In rare and ultrarare decay processes these couplings could turn out to have important phenomenological consequences. Furthermore, Bjorken and Weinberg noted that in the standard Weinberg–Salam gauge theory one can have a naturally occurring coupling of a Higgs particle, a muon and an electron all at one point and hence generating the transition $\mu \to e\gamma$ without extending the number of leptons in nature beyond the famous four.

Before the advent of gauge theories an observation of $\mu \to e\gamma$ would have left us puzzling as to how to proceed. With the great advances of the past few years not only do we know how to tackle the problem but even have several solutions in advance of any signal! Perhaps none of the models is very suasivious. However, if a signal is seen in the current experiments then one must take these ideas seriously and more impressive support for the gauge theories will have been unearthed. That this will happen in the next month or two is many theorists' midsummer night's dream—but it could turn out to be much ado about nothing.

Spin waves in superfluid ^{3}He

from P. V. E. McClintock

ONE of the most intriguing features of the recently discovered superfluid phases of liquid ^{3}He is that in sharp contrast to ^{4}He, the only other superfluid known in nature, they have magnetic properties. It is hardly surprising, therefore, that efforts to understand superfluid ^{3}He have been influenced by making comparisons with more conventional types of magnetic material, attempting to explore both the similarities and the fundamental differences in their properties. One characteristic feature of ordered magnetic solids is that they may support so-called spin waves, a form of collective excitation among the magnetic elements. The possible existence of spin waves in ^{3}He has consequently been a subject of much discussion during the four years which have elapsed since the superfluid phases were discovered by D. D. Osheroff, then working at Cornell University. It is of particular interest, therefore, that Osheroff and his coworkers at Bell Laboratories have now reported (*Phys. Rev. Lett.* **38**, 134; 1977) the first experimental observation of spin waves in the B phase of superfluid ^{3}He.

The magnetism of liquid ^{3}He arises from the magnetic moments carried by the nuclei of the ^{3}He atoms. In ordinary normal liquid ^{3}He in the absence of a magnetic field the nuclei are so weakly coupled to each other that the liquid is completely devoid of any overall magnetic order. When the liquid is sufficiently cooled, however, things become very different: the onset of long range order at the superfluid transition temperature involves the orientation of nuclear spins in definite directions, producing just the sort of ordered magnetic structure which might be expected to support spin waves.

In the case of a magnetic metal, one of the most convenient ways of demonstrating the existence of spin waves is through use of a resonance technique to excite standing waves in a very thin layer of the material. The situation is somewhat analogous to the more familiar one of standing waves on a stretched string whose ends are fixed in position. Unlike the case of the string, however, it is not the position, but rather the direction of the spins which is fixed on the boundaries of the material and which varies coherently through its thickness. Absorption of energy takes place for radio waves of the right frequency to excite a standing spin wave, that is, one such that the thickness of the layer of metal is equal to an integral number of half-wavelengths. Thus, if the rate of absorption of electromagnetic radiation is measured while its frequency is gradually increased, a series of peaks will be seen corresponding respectively to the fundamental, and higher orders of standing spin waves being excited.

The Bell Laboratories group used what in many ways is a very similar technique in their search for spin waves in superfluid ^{3}He. The combination of a strong magnetic field and the presence of a solid surface was used to fix the spin anisotropy axis n, thus defining the directions of the spins at the surfaces of a series of thin laminae of liquid. These were formed between a set of 20 fused quartz plates each about 7×11 mm in area, spaced 210 μm apart. The magnetic field was applied in the plane of the plates. Under these conditions, although n tries to lie parallel to the magnetic field in the bulk liquid between the plates, it is compelled to take up a different fixed direction where the liquid touches the surfaces of the plates. The competition between these effects causes the direction of n to vary smoothly with position in a completely determined way between the plates. Spin waves consist, therefore, of coherent deviations from this equilibrium situation, and may be expected to reveal their existence, as in the case of the thin metallic film, through the resonant absorption of electromagnetic radiation at particular frequencies depending, in this case, on the temperature and on the magnitude of the applied magnetic field. The cell was placed in a compressional cooling apparatus in which the temperature of the liquid was lowered by reducing its volume and thus solidifying some of it, which was able to reach the so-called superfluid B phase which is stable below its transition temperature at about 2 mK.

In the event, a series of striking absorption peaks was indeed found approximately at the frequencies predicted by theory. Although the peaks were broader than ordinary NMR resonances in ^{3}He, they were quite well defined, thus eliminating the lurking possibility that spin waves in ^{3}He might be modes which are overdamped and therefore unobservable. As the authors point out, it is quite possible that the observed width of the resonance maxima is merely an experimental effect arising from small variations in the spacing of the quartz plates. The change with temperature of the resonant frequencies turned out qualitatively to be very much in line with theoretical expectations.

The experimental results are not, however, in close quantitative agreement with theory. However, although the authors' tentative explanation of the discrepancies (typically about 20%) may or may not be correct, there seems little doubt that they were indeed observing spin waves. Further experimental and theoretical work is clearly required, but it seems that spin wave resonance has now been added to the rapidly growing battery of techniques available for elucidating the dauntingly complicated properties of superfluid ^{3}He. ☐

Complementary genetics

from M. J. Hobart

An EMBO Workshop on Complement Genetics was held in Cambridge, UK on 18–22 March, 1977. It was organised by Dr P. Lachmann, MRC Group on Mechanisms in Tumour Immunity, Cambridge.

THE genetics of the complement system were put on the map, both chromosomally and as a topic of general scientific interest, by the discoveries in 1974 by F. H. Allen, S. M. Fu and their colleagues that the genes coding for Factor B and C2 are linked to *HLA*. This had been foreshadowed in 1973 by the work of P. Demant and colleagues showing that the Ss protein, coded within the *H-2* region of mice, was a complement component, subsequently shown by several groups of workers to be C4.

The complement system consists of at least 18 proteins, including proenzymes and inhibitors, whose activity is usually triggered by the binding of antibody to antigen. It is the major humoral effector system for the elimination of bacteria and viruses in vertebrates and is characterised by complex reaction pathways reminiscent of the clotting, fibrinolysis and kinin systems.

There is now good evidence that three complement components are coded by genes located within the *HLA* region: C2, Factor B and C4. The electrophoretic variants of C4 described by P. Teisberg (Ulleval Hospital, Oslo) show clear *HLA* linkage and both G. Mauff (Hygiene Institute, Cologne) and G. Hauptmann (Institut d'hematologie et de transfusion sanguine, Strasbourg) reported their methods which show the same phenomenon with rather improved resolution. These results confirm the earlier evidence for C4 linkage from studies of a deficient patient's family (C. Rittner, Institute of Forensic Medicine, Bonn). *HLA* linkage is now firmly established for C2 polymorphism (C. Alper, Center for Blood Research, Boston). The less common allele of *C2* (*$2C^2$* in the nomenclature proposed) was reported by T. Meo (Basel Institute for Immunology) to be in strong linkage disequilibrium with *HLA* BW15 and *HLA* CW3.

Alper also demonstrated polymorphism of C8 detected by isoelectric focusing and specific functional detection by haemolytic assay. There are at least three structural variants of C8, two of them common. The locus is not closely linked to *HLA*. Two new families with C8 deficient members were described by N. K. Day (Sloan Kettering Institute, New York) and F. Tedesco (Transplantation, Immunology and Blood Transfusion Service, Milan). In both families, there were totally C8 deficient individuals with different *HLA* types, showing that the deficiency genes also cannot be close to *HLA*. Furthermore it was apparent that there is difficulty in ascertaining the heterozygous deficiency states, since in both families, both parents of the propositi had C8 levels within the normal range. Furthermore Alper reported that members of a deficient family heterozygous for the structural variant could nevertheless have low C8 levels. This may explain the discrepancy between these reports and that of Merritt *et al.* (*Third International Workshop on Human Gene Mapping. Birth Defects* **XII** (7) 331, The National Foundation, New York, 1976) which claimed *HLA* linkage for C8 deficiency.

D. Glass (Robert B. Brigham Hospital, Boston) presented a large series of carefully measured cases of heterozygous C2 deficiency. There seems to be a significant association with juvenile rheumatoid arthritis and systemic lupus erythematosus in these patients. J. Soothill (Institute for Child Health, London) and J. Mowbray (St. Mary's Hospital, London) have evidence that there is an increased incidence of heterozygous C2 deficiency in infantile asthma and eczma. However, it was pointed out in discussion that apparently high recombination frequency between C2 and *HLA* observed by Glass is probably due to ascertainment errors inherent in measuring complement levels.

C3, C4, C5 and C8 are known to be composed of more than one polypeptide chain, but in no case is there any evidence that more than one locus is involved in coding for the observed polymorphisms. A possible explanation for this phenomenon is given by preliminary experiments of H. Colten (Children's Hospital Boston) and his colleagues (reported by Alper). They have shown that both C4 and C3 obtained from cell-free synthesis by immunoprecipitation occur as larger than expected chains. Their suggested interpretation of this result is that C3 and C4 (and, by analogy, perhaps C5 and C8) are synthesised as single polypeptide chains which are cleaved before secretion, rather like insulin. Only one cistron will then be involved in coding for the whole molecule and all markers will be alleles, irrespective of the chain on which they are found. Since there are now some 20 variants of C3, all of them apparently alleles, this view has its attractions. Some similar complement components seem to be the products of tandem duplicated genes. This is the case with C2 and Factor B. Both are linked to *HLA* in the same subregion and with no crossovers indentified between them. Both are single polypeptide chains, heat labile and form part of a complex C3 splitting enzyme. C6 and C7 are consecutively acting components and are both single polypeptide chains. They show close linkage to each other as demonstrated by allotyping (M. J. Hobart, MRC Group on Mechanisms in Tumour Immunity, Cambridge). Although C3, C4 and C5 are similar, C4 is linked to *HLA* but the other two components are not. If these molecules are the products of related genes, then they have been duplicated by processes other than (or additional to) tandem duplication.

A remarkable case of combined

subtotal deficiency of C6 and C7 was reported by P. J. Lachmann (MRC Group on Mechanisms in Tumour Immunity, Cambridge). The proband is in good health. The deficiencies of both components segregate together in the grandchildren, which is to be expected if the structural genes for both proteins are involved, since they are closely linked. Trace C6 and C7 activity appear in the proband's serum in the same pattern of bands, different from either normal C6 or C7 when separated by isoelectric focusing. An interesting speculation is that this may be the result of a 'Lepore' fusion of C6 and C7 genes.

At times the Workshop veered from the strict limits of complement genetics, Alper and F. S. Rosen (Children's Hospital, Boston) presented evidence that C3 nephritic factor is an IgG, on the basis of functional detection of clonal electrophoretic variants and on the transmission of such a clone across the placenta. A. Ferreira (New York University) described a new C4-binding protein in mice, the molecular association giving the appearance of *H-2* linkage. Since more than half of the known complement components now have genetic markers, new components are needed if healthy growth of the topic is to be maintained! ☐

Progress in coronaviruses

from David J. Garves

A symposium on Human and Animal Coronaviruses was held at the Agricultural Research Council Institute for Research on Animal Diseases, Compton UK on 24–25 March, 1977, and was organised by Professor A. P. Waterson, Royal Postgraduate Medical School, London, and Dr D. J. Garves, Compton.

THE Coronaviridae comprise a family of morphologically similar viruses that cause a wide variety of diseases in man and other animals. Following the recognition of coronaviruses as a distinct taxonomic group in 1968 (see *Nature* **220**, 650), with virus particles having a peripheral halo in negatively stained preparations, the number of viruses classified in the group and our knowledge of their characteristics has greatly increased. There are still large gaps in our understanding of the diagnosis, pathogenesis, structure and serology of this economically and, potentially, medically important group and attempts were made to fill these gaps at the symposium.

Recent reports of coronavirus-like particles in samples from human and equine enteritis, human nephropathy and feline infectious peritonitis have raised the problem of which criteria should be used to identify a coronavirus. It has been found that coronaviruses are difficult to establish in cell culture and for this reason morphology of the virus particle provides the major distinguishing feature. J. Almeida (Wellcome Research Laboratories) and R. Bingham (Royal (Dick) School of Veterinary Studies, Edinburgh) reported new evidence for the morphology and structure of the internal component of avian infectious bronchitis virus. They showed electron micrographs of virus particles within which flask-shaped structures could be seen, the mouth of the 'flask' appearing to open at the outer membrane of the virus. Whether these structures are present in other coronaviruses and what relation they have to the organisation of the genetic material has yet to be determined.

Recent studies on the polypeptide structures of human and animal coronaviruses have shown that, while the number and size of the structural proteins vary between members of the family, some similar features can be seen. The polypeptide profiles may provide a method to distinguish relationships within the coronaviruses and in this context the apparent similarity between the polyacrylamide gel patterns for avian infectious bronchitis virus (M. R. Macnaughton, Clinical Research Centre, London) and calf diarrhoea coronaviruses (J. Laporte, Thiverval-Grignon, France) was of interest.

The genome of avian and porcine coronaviruses has been shown to comprise single-stranded RNA but whether this has a messenger role, as in oncornaviruses and enteroviruses, or is a complementary strand, as in paramyxoviruses, has not been determined. Our understanding of the strategy of the coronavirus genome is greater, however, following the reports of Macnaughton and D. H. Pocock (ARC, Compton) that avian and porcine coronavirus RNA contains polyadenylic acid tracts. This evidence strongly suggests that the RNA has a messenger function and is yet another feature, together with virion morphology and the genome size, that coronaviruses have in common with the RNA tumour viruses. Bingham reported, however, that attempts to detect a reverse transcriptase in avian infectious bronchitis virus have proved unsuccessful, in line with the evidence from inhibitor studies suggesting that coronavirus replication does not proceed by way of DNA.

D. J. Alexander (Central Veterinary Laboratory, Weybridge) reported on the finding of long term infection of chickens with infectious bronchitis virus and these results, together with the evidence of chronic infection of mice with hepatitis virus and the carrier status of pigs with transmissible gastroenteritis, prompted K. McIntosh (University of Colorado, Denver) to suggest that future work on the human respiratory coronaviruses should consider implications of inapparent, chronic infections. It is possible that Balkan endemic nephropathy may result from such an infection, as K. Apostolov (Royal Postgraduate Medical School, London) reported a possible serological relationship between this disease and a human respiratory coronavirus.

Coronavirus serology is confused at the moment, with cross reactions being detected between human, bovine, murine and porcine viruses. The serological relationship between porcine transmissible gastroenteritis virus and a canine enteric virus is strong and D. J. Reynolds, (Compton) also presented evidence of antibodies to transmissible gastroenteritis in cats. It is possible that the agent in the cat responsible for the very high antibody levels may be associated with feline infectious peritonitis, a possible coronavirus whose morphology and physicochemical properties were presented by A. Osterhaus (Utrecht, Netherlands). The morphology of the purified particles was not characteristic of other coronaviruses and whether this was due to degradation or a different surface projection structure is not known. The agent of feline infectious peritonitis demonstrated the problem caused by the fact that coronavirus identification rests principally on the shape of the surface projection. It was generally agreed that other features including virus structure, the characteristics of the genome and the mode of replication within the cell needed to be determined before any virus could be assigned to the Coronaviridae, although this information is not known for some well accepted coronaviruses. Similarly, more data would be required before any meaningful subdivisions, such as separating the avian from the mammalian members, could be made within the Family.

In the field of coronavirus disease control there seemed to be doubtful correlation between antibody levels in serum and secretions and resistance to infection. It was recommended that more effort should go into investigating cellular and humoral aspects of immunity to diseases caused by coronaviruses. ☐

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articles

Accretion torques in X-ray pulsars

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The character of intrinsic pulse period changes in X-ray pulsars supports the now conventional model for these sources, wherein the X-ray luminosity is derived from the accretion of matter on to a rotating, magnetised compact object, probably a neutron star, and the pulse period changes are caused by its interaction with the accreting matter.

At least nine galactic X-ray sources are known to emit regularly pulsed X rays[1]. It is widely believed that the compact objects in these binary X-ray pulsar systems are rotating, magnetised neutron stars[2]. Black holes are eliminated as candidates for these objects because of the existence of periodic pulsations. One or more of the following arguments tend to rule against white dwarfs as the compact objects in at least seven of these systems: (1) the pulse period is too short to be a plausible surface rotation period of a white dwarf (see, e.g. ref. 3); (2) the observed X-ray luminosity is larger than the maximum luminosity of $\sim 10^{35-36}$ erg s^{-1} that has been predicted by some theoretical models of accreting white dwarfs[4,5]; or (3) the mass is probably above the Chandrasekhar limiting mass (see, for example, ref. 6).

Another means of distinguishing between white dwarf and neutron star models is through an analysis of the rates of change of the intrinsic pulse periods. As matter is accreted on to the compact star, torques are exerted that will tend to spin it up or down. The time required for accretion torques to spin up a white dwarf will, in general, be considerably longer than for a neutron star. We show here that the observed characteristic spin-up timescales for seven X-ray pulsars strongly support the view that in every source (1) the pulse period reflects the rotation period of a compact object (2), the accretion is mediated by a disk surrounding the compact object and rotating in the same sense, and (3) the compact object is a neutron star rather than a white dwarf.

Theory

In the accretion picture of pulsating X-ray sources, material from an overflow of the critical potential lobe of the companion or from a stellar wind is accreted toward the compact star in nearly Keplerian trajectories. If the net specific angular momentum of the accreting matter is too large to allow direct accretion on to the compact object, then matter will accumulate until an accretion disk forms and permits the outward transport of angular momentum through viscous forces. Ultimately, the matter reaches the Alfvén surface: the surface where the magnetic field of the compact object first dominates the particle dynamics, and where the matter will be forced to nearly co-rotate with the rotating compact star, provided that the circular Keplerian orbital velocity at the Alfvén surface exceeds the corotation velocity Ωr_A. Here, Ω is the magnitude of the vector angular velocity $\mathbf{\Omega}$ of the compact star and r_A is the characteristic radius of the Alfvén surface. An approximate relation[7,8] between the Alfvén radius and the parameters of the accreting matter and compact object is given by

$$r_A \approx 3\times10^{8}\,(\xi v_r/v_{ff})^{2/7}\left(\frac{\mu}{10^{30}\ \mathrm{G\ cm^3}}\right)^{4/7}\left(\frac{\mathscr{L}}{10^{37}\ \mathrm{erg\ s^{-1}}}\right)^{-2/7} \times\left(\frac{M}{M_\odot}\right)^{1/7}\left(\frac{R}{10^{6}\ \mathrm{cm}}\right)^{-2/7}\ \mathrm{cm} \qquad (1)$$

μ, M, R and $\mathscr{L}$ are the magnetic moment, mass, radius and luminosity of the X-ray star, respectively. The quantity ξ represents the fractional solid angle subtended at the compact star by the infalling matter at the Alfvén surface, and v_r/v_{ff} is the ratio of the average radial infall velocity of a particle to its free-fall velocity just outside the Alfvén surface.

In the accretion process, angular momentum is transferred to the X-ray star and its rotation period will change. The X-ray pulsations result from the rotation of the compact object, and the fractional rate of change of the pulse period, $P = 2\pi/\Omega$, can be expressed as[7,8]

$$\frac{\dot P}{P} = \frac{\dot M}{M}\left(\frac{M}{I}\frac{\mathrm{d}I}{\mathrm{d}M} - \frac{h_a}{h}\right) + \frac{\alpha}{I\Omega} \qquad (2)$$

I is the moment of inertia of the compact object, and h_a, h and α are the component of the specific angular momentum parallel to $\mathbf{\Omega}$ of the material crossing the Alfvén surface, the specific angular momentum of the X-ray star, and the external (viscous and magnetic) torques acting on the X-ray star just above the Alfvén surface, respectively. If the accreting matter counter-rotates with respect to $\mathbf{\Omega}$, then $h_a < 0$ and the second term in brackets in equation (2) tends to increase $\dot P/P$ (that is, tends to spin down the compact object). The first term in brackets is, in general, small compared to the second term for the case of a white dwarf or neutron star with $r_A \gg R$, provided that an accretion disk exists. The maximum spin-up or spin-down rate will occur when the external torques are small, which is expected if the circular Keplerian orbital velocity at r_A exceeds Ωr_A (ref. 9).

It is instructive to compare the maximum spin-up rate with those measured for the X-ray pulsars. The relation $\dot P/P \propto -(\dot M/M)(h_a/h)$ can be expressed[8] in terms of the parameters of the compact star and the luminosity:

$$\frac{\dot P}{P} \approx -2\times10^{-5}\left(\frac{M}{M_\odot}\right)^{-3/2}\left(\frac{R}{10^{6}\ \mathrm{cm}}\right)\left(\frac{R_g}{10^{6}\ \mathrm{cm}}\right)^{-2} \times\left(\frac{r_A}{10^{8}\ \mathrm{cm}}\right)^{1/2}\left(\frac{\mathscr{L}}{10^{37}\ \mathrm{erg\ s^{-1}}}\right)\left(\frac{P}{1\ \mathrm{s}}\right)\ \mathrm{yr^{-1}} \qquad (3)$$

where R_g is the radius of gyration of the X-ray star. When combined with (1), the relation becomes

$$\frac{\dot P}{P} \approx -3\times10^{-5}\,(\xi v_r/v_A)^{1/7}\left(\frac{M}{M_\odot}\right)^{-10/7}\left(\frac{R}{10^{6}\ \mathrm{cm}}\right)^{6/7}$$

$$\left(\frac{R_g}{10^6\ \mathrm{cm}}\right)^{-2}\left(\frac{\mu}{10^{30}\ \mathrm{G\ cm^3}}\right)^{2/7}\left(\frac{\mathscr{L}}{10^{37}\ \mathrm{erg\ s^{-1}}}\right)^{6/7}\left(\frac{P}{1\ \mathrm{s}}\right)\ \mathrm{yr^{-1}} \tag{4}$$

For simplicity, we write this as

$$\frac{\dot{P}}{P} \approx -3\times10^{-5} f\left(\frac{P}{1\ \mathrm{s}}\right)\left(\frac{\mathscr{L}}{10^{37}\ \mathrm{erg\ s^{-1}}}\right)^{6/7}\ \mathrm{yr^{-1}} \tag{5}$$

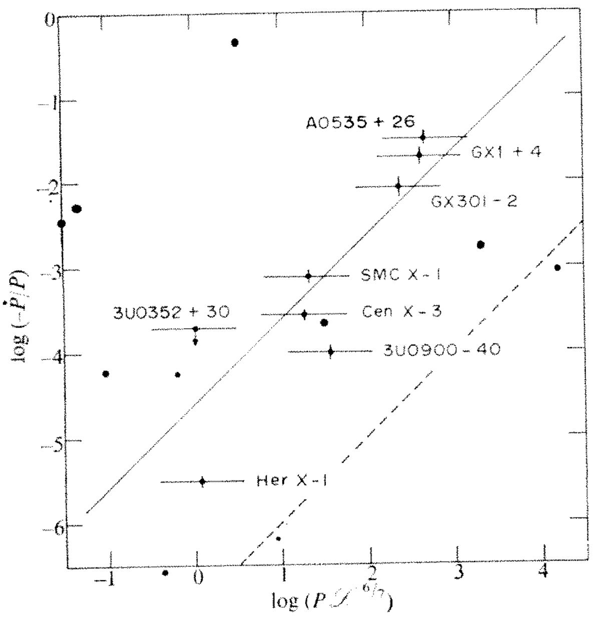
where the dimensionless function, *f*, is expected to be of order unity for a neutron star and contains parameters that are not yet measurable for most or all of the X-ray pulsar systems.

Observational information

The empirical relation between $\dot{P}/P$ and $(P\mathscr{L}^{6/7})$ is plotted in Fig. 1. The luminosities, pulse periods and spin-up rates for eight X-ray pulsars were taken from the sources indicated in the following paragraphs. In each case, the uncertainty in the distance to the source, the possibility of anisotropic beaming of the X-ray flux, and substantial source variability all contribute to the uncertainty in the luminosity determination. We have assigned to each source luminosity a reasonable but arbitrary uncertainty of a factor of three. For four of the sources (3U0352+30, GX301-2, GX1+4, and A0535+26), the identification of the optical counterpart is probable but not certain; if any of these identifications proves to be erroneous, the corresponding distance estimate would, of course, be invalid.

The pulse period for **Hercules X-1** observed with the SAS-3 satellite in 1975 July was 1.2378082±0.0000001 s (ref. 10). This period determination, coupled with Uhuru measurements[11] covering the interval 1971 December–1973 March, yield $\dot{P}/P = (-3\pm0.5)\times10^{-6}\ \mathrm{yr^{-1}}$. Over shorter time intervals, the value of $\dot{P}/P$ fluctuates and its sign even reverses[11]. These sporadic intervals of spin-down, together with the small average value of $|\dot{P}/P|$, are indicative of a near balance between spinup and spindown for this source[9]. Hence, external torques are probably important in this system. The theoretical interpretation of the short-term fluctuations in $\dot{P}/P$ has been discussed previously[12]. The luminosity of Her X-1, taken from ref. 13 (where the distance is estimated to be in the range 2–6 kpc), is $\sim 1\times10^{37}\ \mathrm{erg\ s^{-1}}$. We assume here that a nearly constant X-ray luminosity persists throughout the 35-d cycle of the source (see, e.g., refs 13, 14).

Fig. 1 The empirical relation between the fractional rate of change of the pulse period and the parameter (pulse period × luminosity$^{6/7}$) for eight binary X-ray pulsars. The units of $\dot{P}/P$, P and $\mathscr{L}$ are yr^{-1}, s and 10^{37} erg s^{-1}, respectively. The solid line is the best fit to a power law with logarithmic slope 1.0. The data point for Her X-1 and the upper limit for 3U0352+30 were excluded from the fit. In all cases, we have assumed that the proposed optical identifications are correct (see text). The good agreement with equation (5) for $f \simeq 1$ (see text) lends credence to the presently conventional model of X-ray pulsars as rotating, magnetised, neutron stars that are undergoing accretion. The dashed line is the expected relation for ~1 $M_\odot$ white dwarfs (see text).



The pulse period for **3U0900-40** was measured with SAS-3 in 1975 June–July to be $P = 282.8916\pm0.0002$ s (ref. 6). Ten months later the pulse period measured with SAS-3 was $P = 282.870\pm0.004$ s. This yields an average value of $\dot{P}/P = (-1.0\pm0.3)\times10^{-4}\ \mathrm{yr^{-1}}$. The details of the latter SAS-3 observation will be presented elsewhere. Because only two data points were used to determine $\dot{P}/P$, we cannot estimate the magnitude of short-term fluctuations in this quantity (compare with the discussions of Her X-1 and Cen X-3), and we therefore assign a somewhat larger uncertainty to $\dot{P}/P$ than its formal error. We adopt a value of $\sim 1\times10^{36}\ \mathrm{erg\ s^{-1}}$ for the average luminosity of 3U0900-40 (ref. 15).

Ariel V measurements[16] of the **Centaurus X-3** pulse period in 1975 January (4.83704±0.00004 s) can be combined with Uhuru data[17] covering 1971 January–1972 December, to yield $\dot{P}/P = (-2.8\pm0.4)\times10^{-4}\ \mathrm{yr^{-1}}$. The luminosity is taken to be $\sim 5\times10^{37}\ \mathrm{erg\ s^{-1}}$ (ref. 18). Cen X-3 is also observed to undergo brief intervals of spin-down on shorter timescales[17].

The pulse period of **3U0352+30** ($P = 835.6$ s) has been measured on at least six separate occasions during a 4-yr interval with the Copernicus[19] and SAS-3 (unpublished SAS-3 data, J. G. Jernigan) satellites, and has remained constant to about 1 part in 1.5×10^3. It is possible that a somewhat larger secular change in the intrinsic pulse period has been fortuitously cancelled during these observations by orbital motion, but we regard this as highly unlikely. A luminosity of $\sim 4\times10^{33}\ \mathrm{erg\ s^{-1}}$ is derived from the inferred distance of ~ 350 pc (ref. 20).

The **SMC X-1** pulse period observed[21] with the SAS-3 satellite in 1976 February was 0.714890±0.000004 s. This represents a change of 0.0026 s from the pulse period determined from a reanalysis[22] of Uhuru data from 1971 June ($P = 0.71748\pm0.00026$ s). Hence, $\dot{P}/P = (-7.7\pm2.0)\times10^{-4}\ \mathrm{yr^{-1}}$, where we again adopt a more conservative uncertainty because only two data points were used in the determination. The average luminosity of SMC X-1 (ref. 21) is taken to be $\sim 5\times10^{38}\ \mathrm{erg\ s^{-1}}$.

The X-ray pulsations from **GX301-2** have been studied on five separate occasions by the Ariel V, SAS-3, and OSO-8 satellites during 1975 January–1976 February. In 1975 January the pulse period was 699.3±0.5 s (ref. 23). During the interval 1975 July 18–25 the pulse period ranged from 699.9 to 700.5 (± ~0.2) s (ref. 24). By 1975 December the pulse period had decreased to 696.4±0.4 s (refs 23, 25) and it was observed to be nearly the same in 1976 January[24]. Thus, the pulse period remained the same to within ~1 s for ~0.5 yr after its discovery, after which it decreased by ~4 s during the next ~0.5 yr. To explain this change in pulse period by orbital Doppler shifts, a minimum projected orbital velocity of $v_x \sin i \sim 800\ \mathrm{km\ s^{-1}}$ is required. This source has been identified with the Be star Wray 977 (refs 26, 27); the suggested orbital period is ~ 20 d (ref. 28) which, if verified, would yield a mass function of $f(M) \gtrsim 10^3\ M_\odot$ for $v_x \sin i \gtrsim 800\ \mathrm{km\ s^{-1}}$. Hence, it is highly probable that the observed long-term period changes are mainly intrinsic rather than orbital. We conclude that $\dot{P}/P = (-0.8\pm0.3)\times10^{-2}\ \mathrm{yr^{-1}}$. The luminosity is $\sim 3\times10^{36}\ \mathrm{erg\ s^{-1}}$ for a source distance of ~ 2 kpc (ref. 26).

The periodic nature of the X-ray emission from **GX1+4** was discovered in 1970 October during an MIT balloon observation[29]. The pulse period at that time was 135±4 s (W. H. G. Lewin, personal communication). The pulse period was subsequently measured with the Copernicus satellite[23] in 1972

September (P = 130±0.4 s, aliased by the sampling time of the instrument[23]), and with the SAS-3 satellite[30] in 1975 December (P = 121.370±0.005 s). The pulse period was also measured with the OSO-8 satellite[31] on two occasions: 1975 September (P = 122.46±0.03 s) and 1976 March ($P \sim 120.0$ s). These data yield an average value of $\dot{P}/P = (-2.0\pm0.5)\times10^{-2}$ yr^{-1}. The luminosity of this source is estimated[32] to be $\sim 4\times10^{37}$ erg s^{-1}, based on the tentative identification of an M giant star[33] at ~ 10 kpc as the optical companion (and on its probable proximity, in any event, to the galactic centre).

When pulsations were first discovered[34] from the transient source **A0535+26** on 1975 April 28, the pulse period was 104.17±0.09 s (G. K. Skinner, personal communication). The period was determined about 1 month later to be 103.828 s (ref. 35). We attribute this substantial difference to a change in the intrinsic pulse period. If this is the case, then $\dot{P}/P = (-3.5\pm0.9)\times10^{-2}$ yr^{-1} during 1975 May. Subsequent smaller changes in the pulse period have been attributed largely to orbital motion[36]. The source has been tentatively identified with the Bpe star HDE 245770 (ref. 37). If we take the distance of this source to be in the range ~ 1.5 kpc (J. Hutchings, personal communication) to ~ 5 kpc (ref. 37), then the average luminosity during 1976 May was $\sim 6\times10^{37}$ erg s^{-1}.

The quantities $\dot{P}/P$ and $P\mathscr{L}^{6/7}$ for the above sources are displayed in Fig. 1. Spin-up is observed in all cases (that is, the average value of $\dot{P}/P$ is negative), indicating that the accreting matter generally rotates in the same sense as the compact object (see equation (2)). A log–log plot of relation (5) has slope 1.0 and an intercept at $\log(-\dot{P}/P) = -4.5+\log f$. Superimposed on the data points in Fig. 1 is a best-fit straight line with slope 1.0. The data for Her X-1 were excluded in the fit because of the apparent balance between spin-up and spin-down and the resultant possibility that appreciable external torques are present in this source. In view of the probable fluctuations in f from source to source, the fit is quite good. This result strongly indicates that the second term in parentheses in equation (2) dominates the first term, and that accretion disks are therefore present in these sources. In at last two theoretical studies[38,39], the existence of an accretion disk in some binary X-ray sources had been called into question.

The measured intercept of the straight line on Fig. 1 is -4.6 ± 0.4 (2σ), in good agreement with equation (5) for $f \simeq 1$. Thus, the observationally determined average value of f is $0.3 \lesssim f_{\rm obs} \lesssim 2$.

Discussion and conclusions

For the case where the X-ray star is a rotating white dwarf, the value of f is much smaller than for a neutron star. The radius and radius of gyration of a white dwarf are $\sim 10^9$ cm, while the magnetic moment is

$$\mu = 1\times10^{33}\left(\frac{B}{10^6\ \mathrm{G}}\right)\left(\frac{R}{10^9\ \mathrm{cm}}\right)^3\ \mathrm{G\ cm^3},$$

where B is the surface magnetic field and R is the white dwarf radius. Therefore, we estimate from equation (4) that for a white dwarf $f \sim 0.003$, which is well outside the observationally determined range.

It has been suggested[40] that the short-period ($P < 10$ s) X-ray pulsars may be white dwarfs that are undergoing accretion and are vibrating in a nonradial mode that is excited by the nuclear burning of the accreted matter. In this model, the pulse period is equal to the vibration period of the white dwarf. The model cannot account for the long-period pulsars ($P > 100$ s), however, since the vibration period of a white dwarf cannot be sufficiently long unless its mass were implausibly small ($\lesssim 0.1\ M_\odot$). Thus, if the short-period pulsars are actually white dwarfs, then the strong correlation between $\dot{P}/P$ and $P\mathscr{L}^{6/7}$ on Fig. 1 must be fortuitous.

The experimentally determined range of values for f is consistent with the estimated values of neutron star parameters (that is, $M \sim 1M_\odot$, $R_g \sim R \sim 10^6$ cm, and surface magnetic field $B = \mu/R^3 \sim 10^{12}$ G). Because these parameters appear in the function f with widely varying exponents (see equation (4)), the constraints that one can place on the parameters differ greatly. The magnetic field can range from $\sim10^{11}$ to $\sim10^{13}$ G and the parameter $(\xi v_r/v_{\rm ff})$ can range over ~4 orders of magnitude without significantly affecting our determination of f. The mass and radius, however, should lie within a factor of ~2 of the nominal values for neutron stars suggested above.

The best-fit straight line in Fig. 1 can be used to predict the relationship among pulse period, spinup or spindown rate, and luminosity of any additional X-ray pulsars. For example, if a new X-ray pulsar is discovered and $\dot{P}/P$ is measured, the intrinsic X-ray luminosity of the source can be estimated (unless there are other complications, as in the case of Her X-1) and its distance may then be inferred to within a factor of ~2. Conversely, if the pulse period and X-ray luminosity are known, $|\dot{P}/P|$ may be estimated. We note in this regard that if the existence of 32-min X-ray pulsations in GX17+2 (ref. 19) is confirmed, and if this period represents the rotation period of a neutron star, then $|\dot{P}/P|$ should be ~ 0.7 yr^{-1}. Thus, at the present time the pulse period of GX17+2 should be about one-half its previously reported value, but the spin-up or spin-down timescale of this source would then be considerably shorter than its known lifetime ($\gtrsim 10$ yr). This result tends to rule against a rotational origin for the pulsations in this source. Similarly, if the 4.8 h period in Cyg X-3 is interpreted[41] as the rotation period of a neutron star, then $|\dot{P}/P|$ should be ~ 2 y^{-1}. The measured limit for $|\dot{P}/P|$ in Cyg X-3 is $\lesssim 10^{-5}$ yr^{-1} (ref. 42). Thus, it seems very likely that the 4.8-h periodicity is orbital rather than rotational in origin[42].

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Formation of intense magnetic fields near the surface of the Sun

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Convection concentrates magnetic flux into ropes between granules in the solar photosphere, with average fields of up to 1500 G. The magnetic energy density is locally much greater than the kinetic energy density. Theoretical limits to the amplification of magnetic fields by convection are analysed and related to the observations.

HIGH resolution, ground based observations have recently revealed the presence of very strong, small scale magnetic fields in the solar photosphere[1,2]. More than 90% of the total magnetic flux, outside pores and sunspots, that emerges from the sun is confined to ropes that are only a few hundred kilometres across[1], with typical fluxes estimated to be less than 5×10^9 Wb (ref. 3). Estimates of the field strength vary from 1,000 to 2,000 G: the only direct measurement of Zeeman splitting, in an infrared line, indicates a field between 1,200 and 1,700 G (ref. 2). These magnetic features are closely associated with the cellular convection pattern seen as photospheric granulation. They can be studied indirectly by observing the solar filigree[4] and small scale photospheric faculae[3]. The filigree is composed of bright points and elongated crinkles, which lie in the dark lanes between granules and are moved about by granular convection; moreover, the lifetime of individual features (about 10 min) is similar to the typical lifetime of a granule[4].

In a highly conducting plasma, magnetic flux is inevitably swept aside by horizontal motions, and concentrated into vertical ropes at the edges of convective cells, especially at the corners where several cells meet. The kinematic amplification of weak magnetic fields is limited by ohmic diffusion but stronger fields exert forces which impede the concentration[5-8]. There is, however, no adequate theory to describe the dynamic interaction between magnetic fields and convection in the Sun. Traditionally it has been supposed that flux concentration is halted when equipartition between the local magnetic energy density and the kinetic energy density has been achieved. So the field strength B should be limited by the equipartition field $B_e = (\mu\rho U^2)^{1/2}$, where ρ is the density and U the local maximum speed of convection. In the sun B_e rises from about 200 G at the photosphere to 600 G at 1,000 km depth. Hence the equipartition limit is exceeded by the observed magnetic fields.

There is an obvious upper bound to the average field strength within a flux rope, for the field is contained by the external pressure[9]. Clearly the field at the boundary cannot exceed the value $B_p = (2\,\mu p)^{1/2}$ that balances the external pressure p when there is no gas pressure within the rope. The field may be stronger on the axis of the tube but in a small flux rope the field is nearly vertical and so the average field strength B cannot appreciably exceed B_p. (If B increases with depth the field observed at the centre of the tube may be larger, owing to the greater transparency associated with the Wilson depression.) At the photosphere $B_p \approx 1{,}600$ G and B apparently attains this value. But B_p increases rapidly with depth in the convective zone: at 1,000 km depth $B_p \approx 10^4$ G, at 10,000 km $B_p \approx 4 \times 10^5$ G and at 100,000 km $B_p \approx 10^7$ G.

Thus the small scale magnetic fields pose several theoretical problems. How are they formed and maintained? What limits the field strength? And, in particular, can the field reach values of 10^7 G at the base of the Sun's convective zone?

There are two distinct mechanisms for amplifying the field. The first relies on inhibition of convection by the magnetic field, and the consequent cooling of photospheric gas until thermal and dynamical equilibrium is achieved[10,11]. In the second, flux is concentrated by the motion until the Lorentz force is strong enough to halt the amplification of the field. Both processes must be included in a full treatment of the dynamic interaction between magnetic fields and convection. We shall show that the equipartition limit is irrelevant and that the maximum field strength that can be produced by flux concentration in the Sun is typically an order of magnitude higher than B_e. Near the photosphere, the field is limited by B_p and cooling is important. Deeper down, the magnetic pressure remains small compared with the gas pressure, though fields of the order 10^4 G can be maintained.

The equipartition limit

The principal argument advanced to support the equipartition limit is that flux concentration can only proceed while the magnetic pressure is less than the dynamic pressure of the motion. If the density and temperature within the flux rope are equal to their mean values in the convecting layer outside it then the magnetic pressure must be contained by an excess gas pressure around the flux tube, and fluctuations in the external pressure should be comparable with $\frac{1}{2}\rho U^2$. Hence $B < B_e$. This argument fails, however, if gas can flow downwards along the lines of force, evacuating the flux tube and reducing the internal pressure. Owing to the rapid increase of density with depth in the Sun, the displaced material can easily be accommodated and so the only limit is set by B_p.

Observations show a downward flow associated with small scale magnetic fields. In the photosphere the greatest measured velocity is 2 km s^{-1} and apparently the velocity *decreases* with height (ref. 2). The equipartition argument can be adapted for this flow; Parker[12] has investigated various models with fields that are locally enhanced by motion along the lines of force but they are never increased by more than the equipartition field, which is only 400 G. Moreover, for steady flow along a flux tube the downward velocity should increase with height, contrary to the observations. It seems likely that the downflow is a transient effect, associated with the evacuation of the flux tube as it is formed, rather than a permanent feature of an equilibrium configuration.

The thermal mechanism

Biermann's[10] explanation of sunspots invokes the inhibition of convection by a strong magnetic field. Concentration of magnetic flux reduces the convective energy transport and the photospheric temperature falls since there is less energy to radiate. Cooling proceeds, and the superadiabatic gradient increases, until there is a balance between the reduced energy flux and emission from the photosphere, as well as dynamic equilibrium within the sunspot. This final equilibrium is locally stable[13].

This mechanism suggests that a weak uniform field might be thermally unstable: slight condensations would have lower temperatures and pressures, and therefore be liable to further collapse. A field free gas, however, is not liable to sudden condensations. The gas will remain stable unless the magnetic field exceeds some critical value and its effect on convective transport has become significant. The measured magnetic flux through the solar photosphere corresponds to an average field that is too small to produce this instability. The configuration is metastable and the field must be concentrated to rather greater strengths (for example, by supergranular convection) before any thermal instability can proceed.

There is another stabilising effect. The temperature rises beneath any obstacle that blocks the heat flux: hence a local condensation that increases the magnetic field sufficiently to impede convection tends to enhance the thermal as well as the magnetic pressure[9]. At the level where heat flux is diverted convection must produce additional pressures sufficient to contain any resulting increase of the total pressure in the flux concentration. Since the kinetic energy increases with depth, so that B_e reaches about 10^4 G at the base of the convective zone, it is quite possible to form fields of several thousand gauss in flux ropes that extend deep into the Sun.

These arguments apply to the formation of sunspots, which are large compared with the scale of granular convection. For flux ropes that are small compared with granules, the thermal mechanism cannot consistently be separated from the effect of the convective motion. Slender flux tubes are heated laterally[14], with a consequent reduction in the temperature deficit, and it is unlikely that the thermal instability will grow faster than the rate at which flux is swept across the cell. At greater depths, where the flux rope is approximately in thermal equilibrium with its surroundings, the thermal mechanism cannot function.

The normal photospheric temperature of the Sun is about 6,000 K and a small flux tube may be about 500 K cooler than its surroundings[14]. If the magnetic pressure nearly balances the external pressure then the flux tube must be almost completely evacuated. Below the surface, lateral heating is less important and the temperature difference can increase. Ropes are formed as flux is brought together by granular convection[4,13], giving fields of order B_e, which cause a slight reduction of convective transport in the ropes. The resultant cooling marginally decreases the internal gas pressure. On the axis of a tube, gas is no longer supported by the pressure gradient and therefore falls. The density drops and the tube is squeezed by the external pressure until some equilibrium is reached. A proper description of this process requires a theory of energy transport in the flux tube. We shall instead assume that the density and temperature within the tube adjust themselves to maintain a pressure balance and seek limits to the fields that can be supported by external convection.

Flux concentration by convection

Busse[15] pointed out that laminar convection is dominated by diffusive effects and that the extent to which fields are amplified depends on the relative rates of ohmic, viscous and thermal dissipation. In a Boussinesq fluid (such as a liquid or a shallow layer of gas) the pressure does not enter the equation of state and can be eliminated from the mathematical description of the problem. The equipartition argument, which depends on balancing different contributions to the total pressure, is therefore irrelevant. The governing equations are nonlinear and must be solved on a computer[16]. Several simple configurations have been studied systematically in a series of numerical experiments. In these computations two-dimensional[17,18] or axisymmetric[19] solutions were obtained for convection between free boundaries in a fluid of electrical conductivity σ and kinematic viscosity ν, in the presence of an imposed vertical magnetic field of average strength B_0. The general behaviour of these results, which is similar for each configuration, is summarised in Fig. 1.

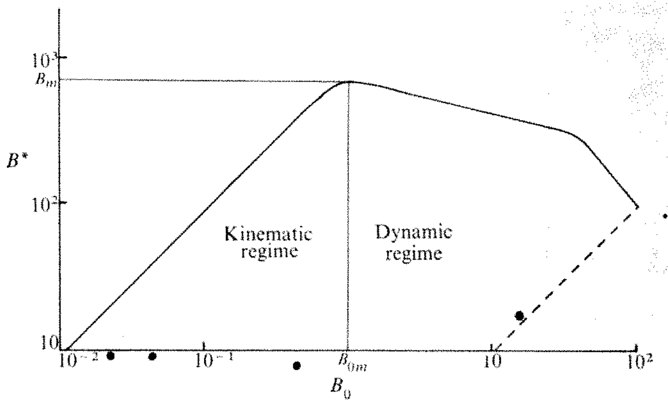

Fig. 1 Amplification of the magnetic field by axisymmetric convection: logarithmic plot of the peak field B^* as a function of the average field B_0. Magnetic fields are measured in terms of the field B_{0m} at which B^* reaches its maximum value B_m. On the broken line $B^* = B_0$. For this particular run $\nu/\eta = 10$, $B_m/B_{0m} \approx 900$ and the magnetic field suppresses convection for $B_0/B_{0m} \geqslant 100$.

When the imposed field B_0 is weak the magnetic forces cannot affect the motion. In a steady state the field is amplified kinematically[6] and the peak field B^* is proportional to B_0. As B_0 is increased, the Lorentz force becomes significant and the convective velocity is reduced. The amplified field B^* reaches a maximum, B_m, when its dynamical effect is important. In this regime the field is confined to ropes, either at the centre or at the edge of the convective cell, and convection is almost entirely excluded from these ropes. An axisymmetric example is shown in Fig. 2: the flux is concentrated at the centre of the cell, which is stagnant, and the convective eddy occupies a toroidal region that encloses the flux rope. This dynamic regime can be successfully analysed and quantitatively understood in terms of a simple physical model[19]. For higher values of B_0 the amplification drops, at first gradually and then more rapidly, and B^* decreases until convection is completely suppressed, whereafter $B^* = B_0$.

In the kinematic regime the work done in containing the magnetic field is small, and the motion is limited by viscous friction. Obviously, kinematic amplification cannot continue after ohmic dissipation has become important. In all the model calculations the transition from the kinematic to the dynamic regime occurs when, or shortly before, the rate of ohmic dissipation in the flux tube becomes comparable with the rate of viscous dissipation in the convecting region. The maximum field B_m can therefore be estimated by equating these two dissipation rates. For higher values of B_0, well into the dynamic regime, viscous losses are relatively small and the rate of working by the buoyancy force is balanced by work done in keeping the magnetic field within the flux rope. With suitably chosen parameters the field $B^* > B_e$, though global equipartition (between the total magnetic and kinetic energies) occurs only at a value of B_0 greater than that corresponding to B_m.

In three-dimensional flow, flux ropes are formed either at the centre of a cell (as illustrated in Fig. 2) or at a corner where several cells meet. The maximum magnetic field can be estimated from a very simple argument. Consider a cell of depth d and similar horizontal dimensions, and suppose that the flux rope has a radius $\delta \ll d$. The ohmic dissipation per unit volume in the

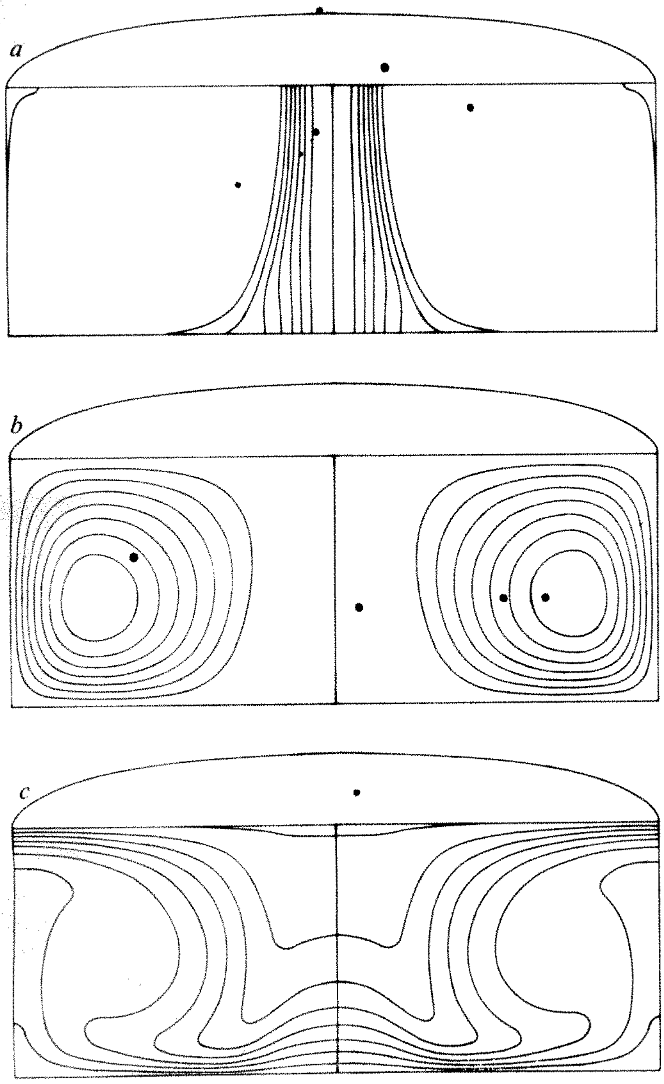

Fig. 2 Thermal convection in a magnetic field: formation of a flux rope in the dynamic regime. Steady solution for axisymmetric flow in a cylindrical cell with free boundaries. *a*, Lines of force of the magnetic field (equally spaced contours of the Stokes flux function); *b*, streamlines (contours of the Stokes stream function); *c*, isotherms, in a meridional plane. The flow is upward around the periphery of the cell and flux is concentrated into an almost stagnant region near the axis.

flux rope is j^2/σ, where j is the current density, and the total ohmic dissipation rate is

$$\left(\frac{j^2}{\sigma}\right)\delta^2 d \sim \left(\frac{B^{*2}}{\mu^2\delta^2\sigma}\right)\delta^2 d = \eta\frac{B^{*2}d}{\mu},$$

where the magnetic diffusivity $\eta = (\mu\sigma)^{-1}$. This expression is independent of δ. The total viscous dissipation rate is $(\rho\nu U^2/d^2)d^3 = \rho\nu U^2 d$. The maximum field B_m is reached when these are approximately equal, so that

$$\frac{B^2{}_m}{B_e{}^2} = \frac{B^2{}_m}{\mu\rho U^2} \sim \left(\frac{\nu}{\eta}\right).$$

This ratio becomes arbitrarily large for appropriately chosen diffusivities. The maximum field B_m is only attained for a particular value, B_{0m}, of B_0. In the kinematic regime, the ratio B^*/B_0 is proportional to the magnetic Reynolds number Ud/η. Hence the transition to a dynamic regime occurs when

$$B_0 \sim B_{0m} \sim \frac{(\eta\nu)^{\frac{1}{2}}}{Ud}B_e.$$

Amplification of magnetic fields in the solar convective zone

In the Sun, convection is not laminar and the pressure cannot be eliminated from the governing equations. Nevertheless, we assume that the field strength cannot exceed the value for which the power needed to contain the flux rope is equal to the rate of turbulent energy dissipation. Observations of granules and supergranules suggest that convective eddies lose their identity after a lifetime τ comparable with the turnover time d/U, as implied by the mixing length theory of convection. Thus the energy dissipation rate in a cell is approximately $\frac{1}{2}\rho U^2 d^3/\tau \sim \rho U^3 d^2$. Although large scale motion is excluded from the flux rope, inefficient small scale convection is still possible within it. The motion is time dependent and mainly vertical, so the field spreads out with an effective diffusivity $\tilde{\eta}$ that is much smaller than Ud (ref. 20). Applying the same argument as before, we find that

$$\frac{B^2}{B^2{}_e} \sim \frac{d^2}{\tilde{\eta}\,\tau} \sim \frac{Ud}{\tilde{\eta}}.$$

Thus the ratio of B_m to the equipartition field B_e varies as the square root of the magnetic Reynolds number. (This would follow directly from the previous result if ν were replaced by an eddy diffusivity of order Ud.) The maximum field $B_m \sim (\mu\rho U^3 d/\tilde{\eta})^{\frac{1}{2}}$ then occurs when the average field through the cell is $B_{0m} \sim (\tilde{\eta}/Ud)^{\frac{1}{2}} B_e$.

To find upper bounds for the field concentrated between granules or supergranules we need estimates of the diffusivity $\tilde{\eta}$. Microturbulent velocities in sunspots have magnitudes of about 1 km s^{-1} and horizontal scales of about 10 km (ref. 21): adopting similar values for small scale convective motion in flux ropes between granules, we have $\tilde{\eta} \sim 10^7$ m^2 s^{-1}. Taking typical values for the granular velocity field ($U = 1$ km s^{-1}, $d = 10^3$ km, $\rho = 10^{-3}$ kg m^{-3}, $\tau = 500$ s) we then find that $B_e \sim 350$ G, while $B_m/B_e \sim 15$; hence $B_m \sim 5{,}000$ G and is greater than B_p. In practice, therefore, the limit is set by the pressure balancing field B_p, which can be reached by purely kinematic amplification, without the magnetic forces having a significant effect on convection. Now B^* will not reach 1,600 G if B_0 is less than about 16 G. Hence strong fields should be present only for fluxes $B_0 d^2 \gtrsim 10^9$ Wb, which is consistent with Mehltretter's[3] estimate of the flux in small scale features.

Magnetic fields are also concentrated at the boundaries of supergranules, which correspond to larger scale and more deepseated convection ($U = 0.4$ km s^{-1}, $d = 1.5\times10^4$ km, $\rho = 10^{-2}$ kg m^{-3}, $\tau = 40$ h). An estimate of the magnetic diffusivity derived from the decay of longlived sunspots gives $\tilde{\eta} \approx 2\times10^7$ m^2 s^{-1} (ref. 20). Hence $B_m/B_e \sim 9$ and, since $B_e \sim 1500$ G, $B_m \sim 12{,}000$ G. This crude upper bound is greater than any field that has been observed on the sun but much less than the pressure-balancing field in the same region. The corresponding flux, $B_{0m}d^2 \sim 10^{12}$ Wb, is comparable to that in a small sunspot.

Deep down in the convective zone the scale of motion is likely to be around 10^5 km. A crude estimate of B_m yields a value of about 70,000 G, corresponding to a flux of about 10^{15} Wb. If the available flux is no more than, say, 10^{14} Wb (the flux through a large sunspot or an active region) then the magnetic field is kinematically amplified to a value around 10,000 G.

We do not suggest that these estimates are precise. But we have shown that fields greater than the equipartition value can be formed where flux is concentrated by the horizontal flow. In the Sun, the convective velocity is outwards from the centres of granules and supergranules and, in a compressible layer, the horizontal velocity is reversed only near the bottom of a convection cell[22]. Hence concentration is predominantly at the cell boundaries. Some field lines are also carried around within the cells but observations of inner network fields[2] suggest that the flux involved is relatively small.

Near the photosphere an initially weak field will be concentrated by convection until the thermal instability takes over. The field is limited by the need to maintain pressure balance, and cannot appreciably exceed 1,600 G in a small flux rope. At depths greater than about 1,000 km thermal effects are less important, cooling is slight and flux concentration is limited by diffusion. The maximum field B_m may be an order of magnitude greater than the equipartition field and we expect that flux ropes may have fields of up to about 10^4 G. Similar arguments may be applied to estimate the fields in other stars where magnetic flux is concentrated by convection.

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Discovery of the Hadrynian Polar Track and further study of the Grenville problem

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Palaeomagnetic poles from North America for the period 1,150–650 Myr fall on a simple single great circle polar track—the Hadrynian Track, and the Grenville Loop. This Loop is consistent with Laurentia and Grenvillia having been an integral single plate unit from the onset of remanence blocking (metamorphic cooling) in the Grenville Province.

PALAEOMAGNETIC studies from the Grenville Province have yielded palaeomagnetic poles that apparently differ from those observed from Interior Laurentia. The many models proposed to explain this discrepancy fall into two classes: the two-plate model [1–6], and the one-plate model[4,5,7–10]. On the two-plate model, it is considered that the north-trending Grenville Track records the gradual convergence of a separate Grenville plate towards Laurentia. Difficulties with the two-plate model are: (1) the magnetisations which are all post-orogenic were reset at the maximum separation of the two regions, the collision itself apparently produced barely any detectable reheating, (2) the lack of evidence of a suture zone, and (3) the presence of pre-orogenic geological units which cross the Grenville Front and can be recognised as far as 100 km into the Grenville Province. On the one-plate model, the Grenville Track is incorporated into the North American polar wander path by means of a so-called Grenville Loop. The difficulty of this model is in establishing the sense and time calibration of this Loop. Both counter-clockwise[10] and clockwise[4,5,7–9] loops have been proposed. Establishing the age sequence of the Grenville Track is clearly of major importance. In comparison with the radiometric evidence, the ages assigned to Grenville Loops seem to be either too old[7,8] (1,400–1,250 Myr), or too young[4,5,9] (800–600 Myr). In addition, some of the reported Grenville poles which do not fall on the so-called Grenville Track (Fig. 2) have not been satisfactorily accommodated by any of the proposed one- or two-plate models.

We present here a polar wander path for the period 1150–670 Myr (Figs 1–3) which uses all the presently available North American palaeomagnetic data. A portion of the path forms a Grenville Loop which incorporates all of the Grenville poles (including a north-trending Grenville Track). It also contains some non-Grenville poles. The age assignment for this loop (1,050–950 Myr) is consistent with radiometric evidence. The latter portion of the polar path (950–670 Myr) follows an almost great circle path (Figs 2 and 3) from close to the Equator at 180°E, over the present geographic North Pole, and down to the Equator again close to 0°E. During all portions of this path the average drift rate relative to the pole is 8 cm yr^{-1}, a much more conservative figure than the drift rates required by the previously proposed one-plate models.

Data interpretation criteria

For the construction of this path some initial interpretations are questioned, and new interpretations are presented. In our opinion it is difficult in any study of igneous, metamorphic or sedimentary rocks to show indisputable evidence of an initial (primary) magnetisation. Only a positive contact test provides evidence of a single simple thermo-remanent magnetisation (TRM). In all other cases, elucidation of the sequence of remanence acquisition can only possibly be achieved by detailed laboratory experimentation. For igneous and metamorphic rocks extensive alternating field (a.f.) and thermal remanence cleaning is obligatory; a.f. cleaning alone is inadequate to identify the remanence carrier (for example, magnetite or haematite). Thermal experiments provide the respective blocking temperature spectra, and it may then be possible, using vector analysis, to establish the magnetisation sequence. This still remains hazardous unless it can be shown that both haematite and magnetite remanences are the result of a single cooling event (TRM). Chemical remanence (CRM) acquisition at low or intermediate temperatures, which is probably commonplace, can quite easily produce haematite remanence with high blocking temperature and high coercive force characteristics, but which is of secondary origin. For sedimentary rocks, especially red beds, thermal experiments alone are insufficient for unravelling complex magnetisation sequences; chemical leaching must also be performed[11]. A.f. experiments are not obligatory but are considered extremely

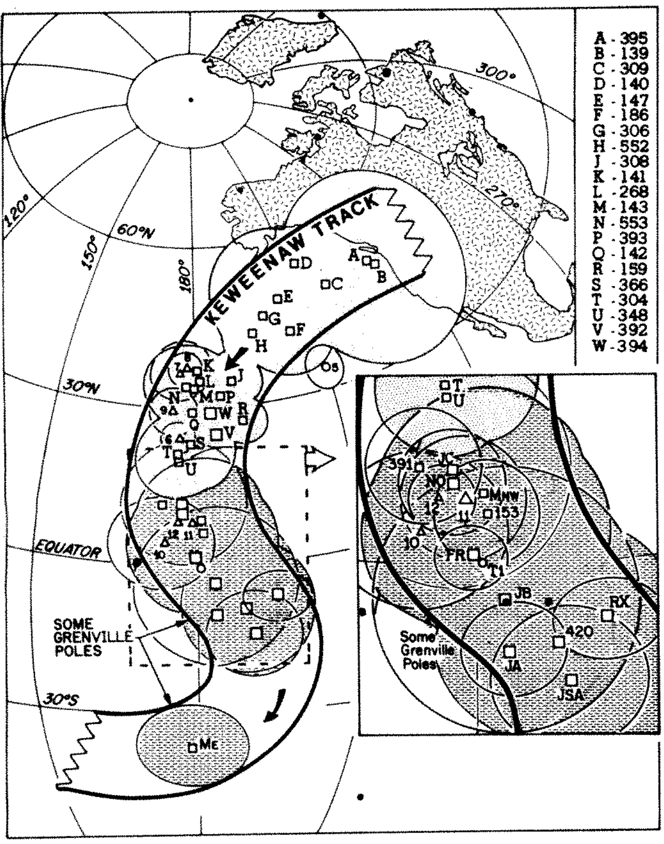


Fig. 1 Polar path for the interval −1,150 to −1,000 Myr-relative to North America. Inset shows detail of the descending portion of the Grenville Loop. Poles A→W and those with three digits are listed by their Ottawa Catalogue number[25]; △, poles (enumerated 5–12) from sequential strata in the Grand Canyon Supergroup[12]; all other poles catalogued by letters are referenced in the text. □, Site unit weight; ○, sample unit weight. Approximate error ovals (95%) for the Keweenawan and Grenville data are represented respectively by the dotted and horizontally hatched clouds, and plotted individually for other results. Lambert equal area projection centred on 180°E, 30°N.

valuable. These requirements might seem stringent, but to understand complex geological processes, palaeomagnetic data of the highest quality possible are required. Applying these criteria to this data set, some poles can be regarded as key reference poles (KRP), shown as the larger symbols in Figs 1–3, and discussed in the detailed formulation of our polar path.

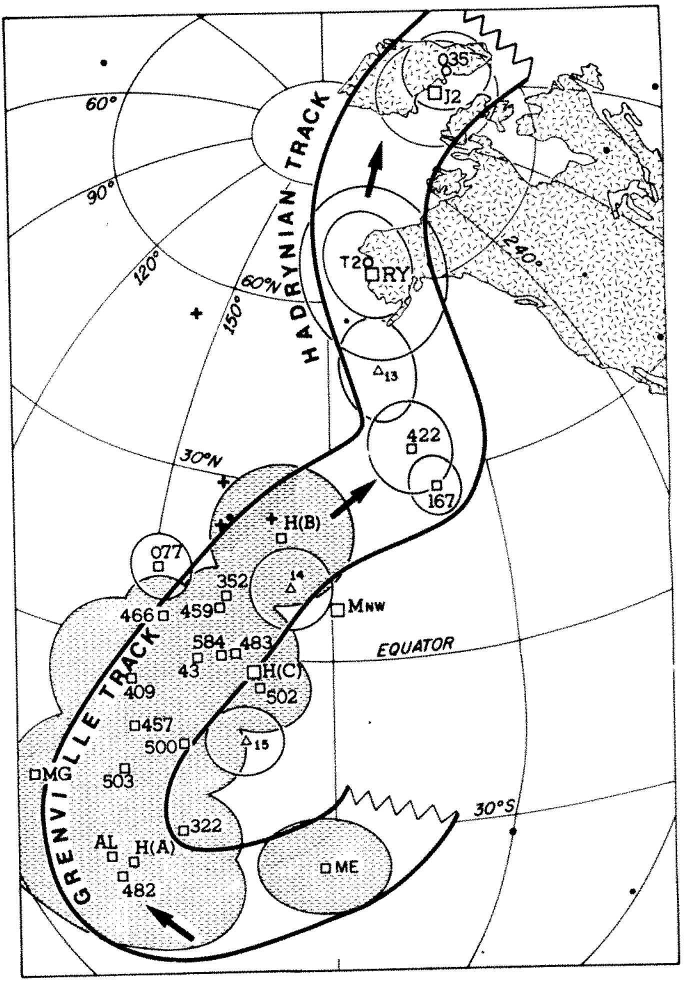
The Grenville Loop

The downward portion of the Keweenawan Track (Fig. 1), which has been established for a few years, is based on a number of KRPs from the Keweenawan volcanic sequences, for example, the Portage Lake Lavas (pole 492) is the mean of several studies. This trend is confirmed by eight poles (5–12) from a measured stratigraphic sequence in the Grand Canyon Supergroup[12]. Pole 11 near the equator from the Cardenas Lavas with a Rb/Sr isochron age of 1,090±70 Myr (ref. 13) is not significantly different from pole 391 (the Rama diabase)[26] suggesting consanguinuity of these intrusive and extrusive units of the Grand Canyon Supergroup.

Extension of the polar path southwards over the equator is based on the evidence of four new poles; the Nonesuch Shale (NO) (ref. 14), the Freda Sandstone (FR) (ref. 14), the Rapitan Group (RX) (ref. 15), and the Jacobsville Formation (JC→JB→JA→JSA) (ref. 16). All these results were obtained after extensive chemical, thermal and a.f. experimentation. The Nonesuch and Freda poles sampled over a 900-m section indicate a southward trend. Pole RX from the Rapitan Group is the direction most persistent after chemical leaching. The Jacobsville was sampled over a lateral distance of 350 km in three areas: Sault Ste Marie (C), Marquette (B) and the Keweenaw Peninsula (A). Although there is no direct stratigraphic control over the relative positions of these three areas, isopach maps[17] indicate the age sequence should be C→A. The pole JSA obtained from nine sites (including a field reversal) brings the polar path to 15°S in the descending portion of our Grenville Loop. It is most interesting that the previously underemployed pole 420 from the Shabogamo Gabbro[8] near the Grenville Front falls between poles RX and JA. From this downward-trending limb the polar path is connected to the southern end of the Grenville Track through the Grenville pole Me (Mealy Mountain—east component)[8].

The north-trending Grenville Track is based on detailed studies of polyphase magnetisations[3–6, 7–8]. In studies with two-phase magnetisations the remanence component with lower blocking temperature, lower coercive force characteristics (Grenville Group B poles) plot to the north of the higher blocking temperature, higher coercive force magnetisations (the Grenville Group A poles). Assuming that these magnetisations record a simple slow cooling exhumation TCRM–TRM, the designated age sequence then would be from south to north. A TCRM thermochemical remanent magnetisation, is to be expected as chemical changes are bound to occur during any extreme reheating (metamorphism) event. Support for this simple TRM hypothesis is provided by the record of A group poles carried by both magnetite and haematite in studies from the Magnetawan metasediments[10], and the Haliburton intrusives[6].

Fig. 2 Polar path relative to North America for the interval −1,000 to −850 Myr. The poles are labelled as before. □, Site unit weight; ○, sample unit weight. Approximate error ovals (95%) for the Grenville data are represented by the horizontally hatched cloud, and plotted individually for other results. Lambert equal area projection centred on 180°E, 30°N.



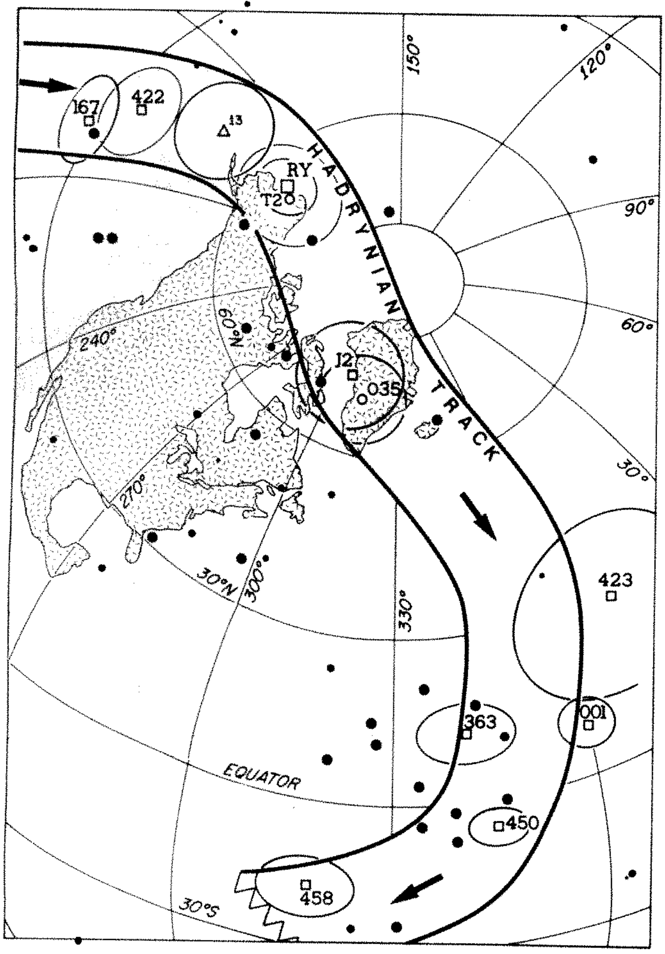


Fig. 3 Polar path relative to North America for the interval −950 to −670 Myr. The poles are labelled as in Fig. 1. □, Site unit weight; ○, sample unit weight; ●, Grenville Province dyke poles; △, Grand Canyon pole (13). Solid plots indicate individual site mean poles from the post-metamorphic Grenville Province dykes[23]. Approximate error ovals are plotted individually. Lambert equal area projection centred on 330°E, 60°N.

Detailed experimental work on the Allard Lake anorthosite (AL) provides particularly good evidence for a simple metamorphic TRM[19]. Only one direction was found but both polarities are present; normal carried by magnetite, and reverse by haemo-ilmenite. Where dual magnetisations of differing remanence directions are ascribed to separate magnetite and haematite remanences, the evidence for the northward shift is less convincing because of the possibility of chemical remanence overprinting (for example, poles 482 and 483 from the Morin anorthosite[18]). By comparison with the Allard Lake result (AL) it is most probable that pole 482 represents a TCRM.

On this polar scheme, poles Me and 420 from or adjacent to the eastern Grenville Province predate poles from the western Grenville Province which define the Grenville Track; this indicates that uplift cooling in the east predated cooling in the west as previously suggested[20]. The Shabogamo Gabbro (pole 420) outcrops outside the Grenville Province, however, and may belong to an earlier portion of the Laurentian Precambrian polar path. Pole Mnw obtained from a.f. experiments alone from the eastern Grenville is ambiguous; it may belong to the descending Keweenaw Track (Fig. 1) or the ascending Grenville Track (Fig. 2). Owing to lack of thermal cleaning, this cannot be resolved at present.

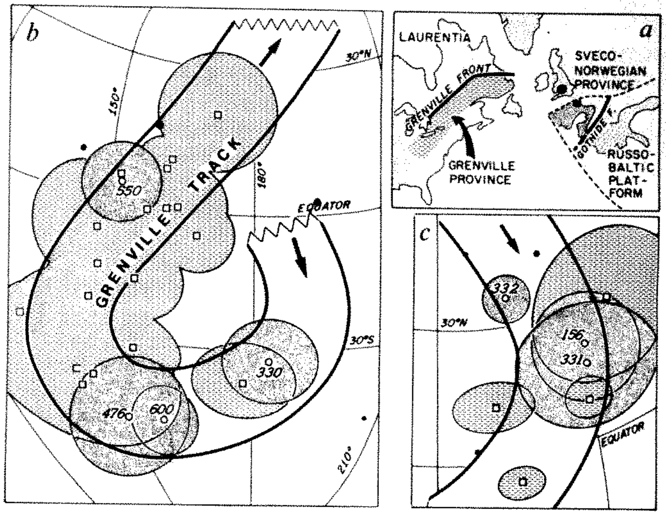
The Hadrynian Track

From about 950 Myr at 180°E on the Equator the polar path moves northwards over the present geographic north pole and then southwards to the 675 Myr Franklin KRP (450) near the Equator at 0°E. Because of the age limits on this path, we suggest the name Hadrynian Track is most appropriate.

Transition from the Grenville Loop to the north-trending Hadrynian Track is suggested by two studies, which also confirm our one-plate model. Firstly, contrary to the original suggestion[6], the magnetisation sequence A→C→B from the Grenville Province Haliburton intrusives favours a one-plate model. On a two-plate model pole (H)B lies well north of the assumed polar juncture (collision) which supposedly took place near the Equator. Also, poles 13–15 from the upper part of the Grand Canyon Suite[12] are consistent with a one-plate model, and a northward-trending Hadrynian Track if the accepted magnetisation sequence is 15→14→13. No chemical leaching was attempted on the ferruginous units 14 and 13, and it is questionable whether the initial magnetisation has been effectively isolated. Furthermore, studies of the probably correlative and contemporaneous haematite-rich Rapitan Group[15] and underlying Little Dal Lava from the Mackenzie Mountains N.W.T. indicate that secondary haematite overprints with similar poles are quite common (for example, pole RY). On the upward trend the Hadrynian Track picks up poles 422 (Stoer Group), and 167 (El Paso) both of which are dated at approximately 950 Myr, and the secondary site mean poles of the Pikes Peak Granite[21] (crosses).

For the high latitude portion of the Hadrynian Track the KRPs are RY (Rapitan Group) and J2 (Jacobsville Formation). Both these magnetisations have high blocking temperature and remanent coercive force spectra. Chemical experiments on the Rapitan indicate that RY most probably postdates RX. J2 is a CRM superimposed upon the JA, JB and JC magnetisations. Supporting evidence for the track over the pole is provided by poles Orienta 2 (T2) and Chequamegon (035) (ref. 22) which plot near the RY and J2 poles, respectively. The Orienta 1 (T1) and Orienta 2 (T2) have great similarity with JA and J2. Site poles from Grenville Province diabase dykes[23] which postdate the Grenville metamorphic episode can be divided into two groups, one tightly grouped about the Franklin pole (450), the other group yielding poles which crudely follow the Hadrynian Track. On the downward portion of the polar path, the track passes sequentially through poles from the basal Torridon sandstone (423), the Torridon Group (001), the Fron-

Fig. 4 Comparison of poles from the Sveco-Norwegian and Grenville Provinces. *a*, The proposed reconstruction as discussed in the text, showing the Gothide and Grenville Fronts subparallel. *b*, After correction to this reconstruction poles 330, 600, 476 and 550 from the Sveco-Norwegian Province gneisses are not significantly different to poles (hatched cloud) from the Grenville Province. *c*, Poles 332, 156, 331 from late-stage dykes intruding the Sveco-Norwegian Province are consistent with contemporaneous poles (horizontally hatched) after correction to the proposed reconstruction.



tenac dykes (363), and terminates with the Franklin diabase (450) and the Tudor Gabbro (458). Placing of the latter pole on this portion of the track is in accordance with the reported K–Ar isochron age[24].

Grenville and Sveco-Norwegian Provinces

The proposed Grenville Loop accommodates all the relevant palaeomagnetic data available including Grenville poles which lie outside the Grenville Track, and the non-Grenville poles which fall close to the Grenville Track, thereby seriously favouring a one-plate model for development of the Grenville Province. Because of the geologic and radiometric similarities of the Sveco-Norwegian Province of the Baltic Shield, it is pertinent to investigate whether palaeomagnetic poles from this region are compatible with the above polar path. Previous comparisons of poles from the Grenville and Sveco-Norwegian Province have explained the residual polar discrepancy after correction to the Bullard refit in terms of transcurrent motion along the line of the Caledonides[5]. This method does not provide an exact polar match; the Grenville and Sveco-Norwegian Tracks are still seen to cross-cut one another. As shown in Fig. 4 an exact match of the poles can be achieved by a 30° clockwise rotation of the Baltic Shield about a Euler pole at 170°E, 40°S after first correcting to the Bullard reconstruction. Poles 330 and 476 (Rogaland basement) and pole 600 from the Farsund intrusion[27] plot near the base of the Grenville Loop, while pole 550 (Bamble area)[28] plots close to the end of the Grenville Track. Poles from the Hunneadalen dykes (332) and Egersund dolerites (156, 331) which have been dated by K–Ar at approximately 660 Myr are shown to bear the same relationship to the Sveco-Norwegian Province as the Grenville Province diabases do to the Grenville Province. In this configuration the Gothide Front, which is sub-parallel but not colinear with the Grenville Front, is seen to form the southern margin of a unified Grenville–Sveco-Norwegian Province. The Grenville Front forms the northern boundary. This reconstruction further confirms our case for an integral single plate unit consisting of the Grenville–Laurentian, and Sveco-Norwegian–Baltic Provinces during the remanence blocking, caused by metamorphic cooling in the Grenville and Sveco-Norwegian Provinces. In conclusion, however, it does in itself pose another fundamental question: what mechanism was responsible for the intense remobilisation of the Grenville Province between 1,050 and 950 Myr?

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letters to nature

Detection of optical pulses from the Vela pulsar

PULSED optical emission has been detected from the Vela pulsar, PSR 0833-45. Double pulses were detected from an area which included Lasker's[1] star M. The pulsed emission has a time-averaged blue magnitude of approximately 25.2 min.

The observations were made on three nights, 24–27 January, 1977, at the *f*/15 focus of the 3.9 m Anglo-Australian Telescope, Siding Spring, Australia. A twin photometer with two apertures, each 5.0″ arc in diameter, spaced by 17.5″, was used for all observations. Its two photomultipliers had S-20 photocathodes and were used without filters. The signals were fanned out to three experiments which were made as independent as possible.

In the first pulse, counters connected to each photomultiplier were sampled every millisecond by the telescope's instrument computer system, the data recorded on magnetic tape, and simultaneously binned at the apparent pulsar period for on-line display. The UTC of each sample was established with an error in epoch of ±1 ms and in frequency of one part in 10^9 using shortwave radio time-signal transmissions. The candidate position was interchanged from one aperture of the photometer to the other at intervals of about 1 h. Pointing and guiding were estimated to be reliable to 1 arc. As a check on our techniques, the Crab pulsar also was observed each night.

In off-line analysis the magnetic tape data were synchronously integrated at the apparent pulsar period in blocks of about 1 h duration. These profiles were then added in the correct phase by computing arrival-times at the solar-system barycentre. Data for the pulsar barycentric period used in this analysis were obtained from the continuing series of pulsar timing observations[2] at the NASA Deep Space Station, Tidbinbilla, at a frequency of 2295 MHz. Radio observations were made before and after the optical observations.

Table 1 Observations in Vela field

No.	α(1950) h min s	δ(1950) ° ′ ″	Integration time (h)	Description
1	08 33 39.35	−45 00 08.6	6.34	Radio position from ref. 4
2	08 33 39.26	−45 00 11.7	1.85	Position of star M as given in ref. 1
3	08 33 39.35	−45 00 06.6	1.17	2″ arc N of radio position[4]
4	08 33 39.43	−45 00 13.2	0.33	0.07 s E of the star B2 0.8″ E, 4.6″ S of radio position[4]

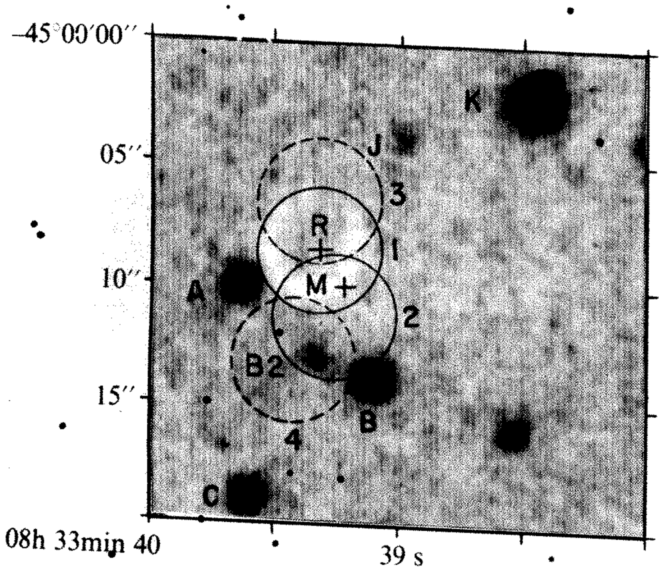

Fig. 1 The field of the Vela Pulsar as it appears, shear distorted, on the TV monitor of the Anglo–Australian Telescope. Circles 1 and 2 enclose the areas of sky where pulses were detected. The radio position (R) of Goss *et al.*[4] and the position of star M are indicated by crosses. No pulsed light was detected from the areas of sky enclosed by the dashed circles 3 and 4. The white circle enclosing the radio position is a fiducial mark in the TV monitor.

In experiment 2, the data were integrated on-line in a Fabri-Tek signal-averager at the appropriate Doppler-shifted period. The period was generated by a Monsanto frequency synthesiser. The start of each run was initiated by the observatory clock but thereafter the frequency synthesiser ran on its own internal oscillator. The third system fed signal and timing pulses to an analogue tape recorder.

Astrometric measurements made from an AAT prime focus plate of stars in the Vela field agreed with those of Lasker *et al.*[3], for their stars A, B, C, F, G and K, but disagreed with the positions given by Lasker[1] for stars A and M. Our position (1950.0) for star A is α = 08 h 33 min 39.68 s±0.03 s, δ = −45°00′ 09.9″±0.3″, and for star M (measured from the photograph by Lasker[1]), α = 08 h 33 min 39.22 s±0.03 s, δ = −45°00′ 10.1″±0.33″.

A total of 10 h observation was obtained at the four positions listed in Table 1 and shown in Fig. 1. Pulses were observed only at the radio position[4], which has errors of Δα = 0.04 s, Δα = 0.8″, and at position 2. Figure 1 shows, with our measurement of its position, that Lasker's star M was within the overlap of the apertures for both of these. Light curves for the observations are shown in Fig. 2, together with the curve obtained by summing the data from the two positions. Figure 3 shows a single curve from the multi-channel analyser system, which gives results in close agreement. The phase of radio pulse (corrected for delay due to dispersion) and γ-ray pulses[5] relative to the optical pulse are also indicated in Fig. 2. Unlike the Crab pulsar, the Vela pulsar emissions in different wavelength regimes are at different phases. The optical pulse shape is double peaked with a component separation of 22±2 ms. The second pulse is stronger, but further observations are required to investigate detailed pulse structure. The radio pulse which is essentially single-peaked, is emitted 20±2 ms before the first optical pulse. This places the optical pulses symmetrically between the γ-ray pulses, which have a separation[5] of 35 ms.

The fact that Lasker's star M was within the aperture at both positions 1 and 2 where pulses were detected allows the possibility that this is the pulsar. Some support for this is given by the slightly greater intensity of the observed pulses at position 2 for which the star M is closer to the centre of the aperture. (The seeing during the observations was generally between 1″ and 2″.) Lasker finds that star M has a B-magnitude of 23.7 with an assumed error of ±0.5. We estimate the time-averaged blue magnitude of the pulsed emission to be 25.2 with the same assumed error. This estimate is based on comparison with the Crab pulsar[6], which has a B-magnitude of 17.0. We assume that the Vela pulsar was wholly within our diaphragm for all observations at positions 1 and 2 and that it has the same colour as the Crab pulsar. Measurements of the B sky brightness at the South Celestial Pole by the UK Schmidt Telescope Unit on the three nights of our observations imply a similar magnitude for the Vela pulsar. At face value,

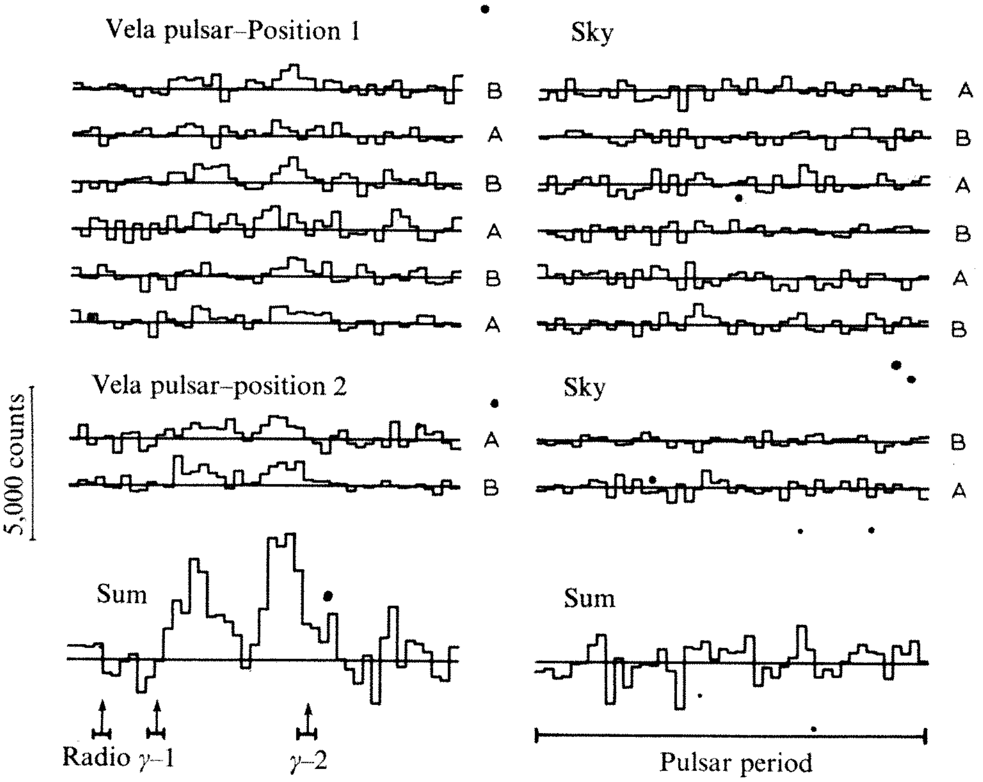

Fig. 2 Phase plots of the light curves of the pulsar (left) and simultaneous sky noise (right) as seen through the twin apertures A and B. Data have been binned to 2 ms resolution. Position 1 is centred on the radio position[4], and 2 on Lasker's position[1] for star M. Phases of emission of the radio pulse[2] with respect to the optical pulses, and of the two γ-ray pulses[5] with respect to the radio pulse are indicated at the foot of the figure. The indicated levels of the background were determined by averaging the baseline regions outside the optical pulses. For the summed pulsar profile the background was 334,800 photocounts per 2 ms bin. The corresponding level of the sky channel was 319,300.

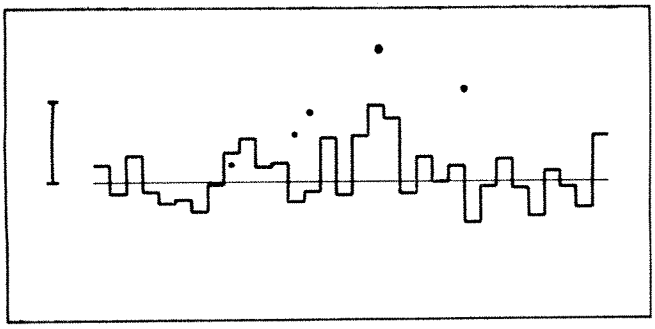

Fig. 3 The first hour long run with the second experiment, on the radio position, displayed in 32 bins. In common with the first experiment every comparable run shows the double peak, subject to photon statistics. The vertical bar is twice the Poisson dispersion.

and assuming conventional values for the distances[7], these magnitudes imply that the dependence of pulsed luminosity on period for optical pulsars follows a P^{-12} law (compare with ref. 8).

Further observations are required to determine whether or not star M is the pulsar and, if so, what fraction of its light is pulsed.

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Search for γ-ray bursts with coincident balloon flights

WE report on an experiment to search for cosmic γ-ray bursts of the type discovered by Klebesadel *et al.*[1], but of smaller size and of sufficient frequency of occurrence to be detected during a 1-d observation program. Two similar detectors, successfully balloon-borne from launch sites in South Dakota and Texas, achieved about 20 h of simultaneous operation at several millibars atmospheric depth, with continuous separation of over 1,500 km on 10–11 May 1975. Fluctuations of the counting rates of >150 keV photons with temporal structures from microseconds to several minutes were compared in order to detect coincident or associated responses from the two instruments. The results of this experiment can be summarised as follows. First, no coincident γ-ray burst events were detected. Second, the resulting integral size spectrum of small bursts, from this and from all other searches, remains a spectrum of upper limits, consistent with an extrapolation of the size spectrum of the largest known bursts, fitting a power law of index −1.5. Third, a curious effect was found which can be described as consisting of associated, but not coincident, counting-rate increases of photons of energy >150 keV. This phenomenon undoubtedly presents a background, event-confusion problem for any single γ-ray burst instrument, placed either on balloons or on satellites which orbit the Earth beneath the trapped radiation.

This experiment was designed to investigate the possibility that cosmic γ-ray bursts could be detected in the size region of 10^{-7} to several times 10^{-6} erg cm^{-2}, below the apparent bend in the size spectrum of Vela events as described by Strong and Klebesadel[2]. It has generally been assumed that the observed bend could be due to instrumental effects[3–5] (because the detector thresholds do not produce a sharp cutoff in event selection, due to the widely varying temporal structures of the bursts). An intrinsic bend in the true size spectrum, however, would be of great interest. A plot of $N(>S)$, the number of events of size greater than S, as a function of event size S, would vary as $S^{-1.5}$ for an extended volume of source objects positioned essentially isotropically, but as S^{-1} for a disk-shaped region and as $S^{-0.5}$ for a one dimensional source region. Thus, the shape of the observed size spectrum not only could indicate a galactic structure in the γ-ray burst source pattern, but also might directly calibrate the intrinsic source strength distribution from the known galactic dimensions.

Our first experiment to search for small γ-ray bursts was the balloon-flight of a single large detector in May 1974—a number of candidate events were found, having sizes consistent with −1.5 index power-law extrapolation, but which could not be established as true γ-ray bursts without confirmation from another detector[3,6].

To obtain a more definite signature for cosmic γ-ray bursts, the dual balloon experiment described here was carried out in 1975. Two similar detectors were flown simultaneously with adequate geographical separation to avoid local cosmic-ray or magnetospherically induced coincident responses. One balloon was launched at 0145 UT on 10 May from Palestine, Texas and floated until about 0400 UT on 11 May. The other balloon was launched about 0340 UT on 10 May from Sioux Falls, South Dakota and floated until 2125 UT on 11 May. The distance between the balloons was always greater than the distance between the launching stations, which is about 1,300 km. The total time of simultaneous float above 6.5 mbar residual atmospheric pressure was about 18 h, most of which was above 3.5 mbar depth.

The detector in each instrument consisted basically of a plastic scintilator 8,900 cm^2 in area and 5 cm thick, viewed by two 5 inch PM tubes in coincidence. The absolute energy threshold was at ≈150 keV, although calibration with radioactive sources showed that the average effective threshold was nearly twice this figure. Particles were excluded by a second, anticoincidence threshold set at 2.5 MeV. In each instrument a time interval analyser (TIA) (ref. 7) was used, measuring the time interval ΔT between each pair of successive photon counts. Thus, rapid, short countrate increases (fast bursts) could be evident in a rate against ΔT histogram as bumps in the region of small ΔT, well separated from the main, Poisson distribution of background γ-ray counts. This made possible a search for temporal structures in counting rates from the 40-ns region continuously to the several hundred millisecond region, with longer structures evident from the use of the total counting rate histogram. The advantage of this system over the conventional counting rate histogram scheme is that orders of magnitude lower bit-rate telemetry can be used. Absolute time coincidences between the two detectors would have been attainable to 72 ms resolution, except that an electronic problem resulted in an actual accuracy of only 1 s. Therefore, coincidences of increases of less than 1 s duration could only be inferred from their likelihood of occurrence in coincident data samples.

The data analysis showed that indeed non-Poisson counting rate excursions were detected with time bases from the microsecond region to the 1-min region. These could be due to local background such as cascade showers, neutron and cosmic-ray interactions and induced radioactivity, and to magnetospheric effects. A phenomenological data treatment was therefore developed, consisting of plots of the number of fluctuations against size in standard deviations, against time base on a logarithmic scale. For each of the two instruments, a smooth, three-dimensional distribution was found to contain only one bump, other than the background distribution having a maximum near the origin. In each case this extra population consisted of very long, one half to several minute, counting rate increases of many standard deviations value. No extra population was found in the 0.1–20 s region where all known γ-ray bursts occur. All increases of over a few standard deviations from each instrument of any time character from microseconds to minutes were compared, however, to search for correlated increases from the two detectors. The results were that, except for those expected on a random coincidence basis, none occurred other than the very long time-base population found with the plots, as described above.

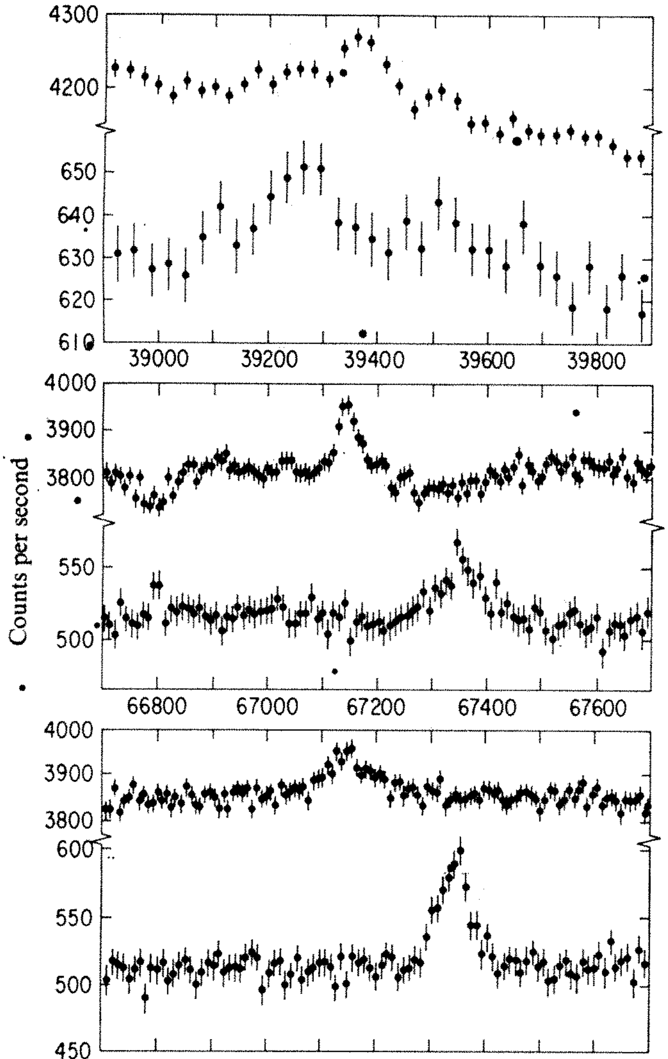

Fig. 1 Counting rate increases exhibited by the γ-ray counters in three occasions. In each record Sioux Falls data are uppermost with Palestine data below. The lack of simultaneity of the events in each case disproves cosmic origin; the fact that the pairs of increases are always separated by a short time delay relative to the time between their occurrence argues for a magnetospheric origin. Such transient increases present a background problem for near-Earth γ-ray burst detectors.

Time histories of the most significant counting rate increases are plotted in Fig. 1*a–c*. (The sensitivities of the two instruments are not the same, but, due to a minor malfunction, the rates of Palestine instrument are somewhat less, and are switched off for 5 s out of each 10 s.) If these increases were γ-ray bursts, assuming the spectrum found typical of longer events[8], their energy content would be around 10^{-5} erg cm^{-2} each. They are clearly not coincident, however. The light travel time between the two detectors is only 5 ms, and yet the temporal separation is many tens of second. The increases are surely associated, however, since there are only several of these events in either tabulation of single counting rate histograms within the 18 h. Thus, the probability is negligible that each pair should fall within a few minutes separation on a random basis. One explanation of this phenomenon may lie in ionospheric or magnetospheric activity with a slowly travelling disturbance centre. Artificial sources are a less likely explanation. We have checked the VLF receiving records of the Naval Observatory in Washington, D.C. Records were available for the VLF stations WLK, Jim Creek, California, and NBA, Balboa, Canal Zone, and for the Omega stations Trinidad and LeMoure, North Dakota. None of these records show any disturbance during the day of 10 May during which three of our events occurred. The responsible electron fluxes would have to be small, less than one per cm^2, such that, if magnetospheric, they lie outside the domain of typically studied trapped radiation phenomena. Such counting rate increases are clearly a danger to any experiment seeking to measure γ-ray bursts without confirmation. In either single balloon flight, these events could have been taken to be exceptionally long γ-ray bursts, were it not for the lack of coincident information in the other. It must be added that one associated event took place near local midnight, so it could not be of solar origin (solar flares would also, of course, be seen as exactly coincident). Thus the need is evident for more sophisticated techniques to search for small γ-ray bursts, a need that applies to low-orbiting Earth satellites instrumentation, as well as to balloon flights.

Figure 2 shows the upper limits to the size spectrum of γ-ray bursts resulting from the analysis of our data. We have included 95% upper limits on the basis that we did not see any actual events in our 1975 flights; the energies where we put our upper limits depend on the time increment used for the burst search. The left mark is the result of a separate 0.1-s search. Since the 0.1-s events are a fairly rare subgroup of events in the total amount of satellite data[9], we regard the results obtained by balloons to date as inconclusive so far as the slope of the size spectrum is concerned. We feel that this unfortunately remains true when the other balloon data, as plotted on this graph, are included. A recent note[10] indicates our reasons for interpreting the results of another balloon experiment search[11] as also consistent with no size spectrum slope change. That note enumerates a number of factors which contribute to rendering a balloon-borne search for bursts less efficient than it may have been intended to be. Our conclusion from this experiment, and from various others[12–14] is that we need an increase in sensitivity by another order of magnitude to be able to adequately detect small γ-ray bursts. If that

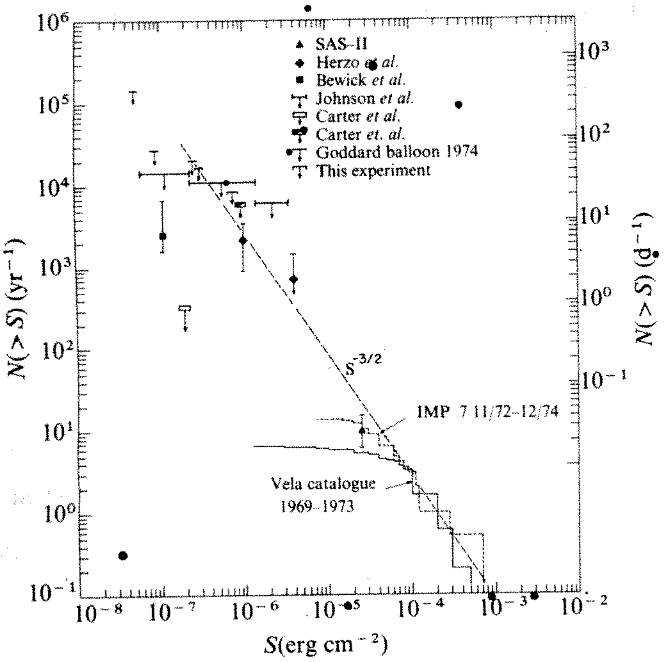

Fig. 2 A plot of the size spectrum of known, large γ-ray bursts and of the various upper limits to the intensity of small bursts, obtained from this and other balloon-borne searches.

can be done, the next step, observing a source direction anisotropy, may be possible.

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Possible γ-ray line from the Crab Nebula

A BALLOON-BORNE γ-ray telescope was flown on 10–11 May 1976 from Alamogordo, New Mexico to search for celestial γ-ray lines in the energy range 100 keV–5.0 MeV. We report here evidence for a line feature from the Crab Nebula at 400±1 keV. Although the counting statistics do not allow us to make an incontestible claim, the fact that it occurs at a reasonable energy to be gravitationally redshifted positron annihilation radiation occurring at the surface of the Crab Pulsar, and in all respects seems to be a real astronomical line, has encouraged us to bring this to the attention of the astronomical community.

The design of the telescope is described in the legend of Fig. 1. A total of 7.4 Crab target and 7.4 Crab background segments were obtained from an altitude of ~ 122,000 ft with zenith angles of 12–57°. An additional 27 segments were obtained from other astronomical sources. The pointing accuracy of the system was about ±1° with the effective entrance aperture increasing slowly with energy from 11° FWHM at 59 keV to 13° FWHM at 1.33 MeV.

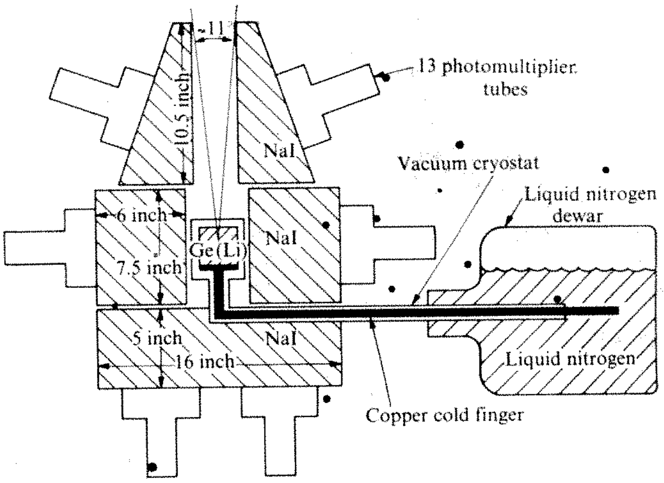

Fig. 1 Schematic diagram of the apparatus. The telescope was designed for optimum sensitivity to lines and contained a 4.70-cm diameter by 6.35-cm long lithium drifted germanium Ge(Li), detector surrounded by 400 pounds of active NaI operated in anticoincidence with the central Ge(Li) detector. The NaI served to collimate the Ge(Li) detector, shield it against atmospheric γ-rays and insure that only full energy events are recorded. Ge(Li) pulses not vetoed by a coincident shield pulse pass through a preamplifier, a shaping amplifier and finally are stored in the onboard memory of a 4096 channel pulse height analyser. The energy resolution of the system below 2 MeV was 3.4 keV FWHM. Data were accumulated in alternate 20-min target-background segments, with the telescope rotated in azimuth 180° but maintained at the same zenith angle for the background measurements.

In Fig. 2 the Crab Nebula difference spectrum extrapolated to the top of the atmosphere is plotted, clearly showing the detection of the X-ray–γ-ray synchrotron continuum at energies below several hundred keV. Also shown is our best fit power law which seems compatible with other recent measurements[1,2]. Beyond 1 MeV the quality of the continuum data has degenerated to the point that the spectrum becomes

Table 1 Results of 3σ line search of Crab target data

Energy keV	σ	Flux (counts s^{-1})	Tentative identification
139*	29.15	$1.29 \pm 0.04 \times 10^{-1}$	^{74}Ge (n,γ) ^{75}Ge
175	5.53	$1.29 \pm 0.23 \times 10^{-2}$	^{70}Ge (n, γ) ^{71}Ge
198*	40.60	$2.28 \pm 0.06 \times 10^{-1}$	^{70}Ge (n, γ) ^{71}Ge
238	3.52	$6.41 \pm 1.82 \times 10^{-3}$	
356	5.00	$8.64 \pm 1.73 \times 10^{-3}$	^{56}Fe (n, γ) ^{57}Fe
400	4.04	$6.31 \pm 1.56 \times 10^{-3}$	Crab Line
440	4.41	$7.37 \pm 1.67 \times 10^{-3}$	^{23}Na (n, n') ^{23}Na
473	4.47	$7.47 \pm 1.67 \times 10^{-3}$	^{23}Na (n, γ) ^{24}Na
497	3.41	$5.60 \pm 1.64 \times 10^{-3}$	
570*	17.15	$4.51 \pm 0.26 \times 10^{-2}$	^{207}Bi (EC) ^{270}Pb
843	3.49	$4.25 \pm 1.22 \times 10^{-3}$	^{27}Al (n, n') ^{27}Al
1063*	23.29	$7.01 \pm 0.30 \times 10^{-2}$	^{207}Bi (EC) ^{207}Pb
1770*	7.10	$7.21 \pm 1.02 \times 10^{-3}$	^{207}Bi (EC) ^{207}Pb

*Lines used in transforming analyser spectra to energy spectra.

Apart from the line at 400 keV, all lines appear with comparable statistical significance in the Crab background spectrum and with enhanced significance in the composite spectrum.

consistent with zero net counts from the Crab. Manipulations of the raw data made in obtaining this spectrum deal with the effects of atmospheric attenuation, detector efficiency, system dead time and drifts in the calibration of the energy axis during the course of the flight. Atmospheric attenuation corrections were based on altitude measurements provided us by the Air Force Geophysical Laboratory Balloon Group, using a mean standard atmospheric model confirmed by rocket measurements on flight day. Photopeak detector efficiency as a function of energy was obtained from elaborate Monte Carlo calculations confirmed by laboratory measurements at 14 discrete energies. The high effectiveness of the anticoincidence shield in suppressing the Compton tail and the steepness of the energy spectrum justified the neglect of down-scattered photons. Dead time corrections based on careful laboratory measurements were generally of the order of 20%, due primarily to veto counts in the NaI shield. To correct for system drift in transforming from pulse height analyser channel spectra to energy spectra we first determined the peak channels, in each record, corresponding to five lines at known energies which appeared clearly in all records. Energies corresponding to other channels were then determined by linear interpolation or extrapolation.

We made a careful search of the data for potential sharp line features from the Crab Nebula, by searching both the target and the background data for 5-keV wide bins which contain > 3σ excess number of counts over what would be expected based upon the two 10-keV wide bins on either side of the 5-keV wide bin in question. A total of 12 lines were detected which appear in both the target and background data. These are listed in Table 1 with some tentative identifications. Apart from the ^{207}Bi lines, which are from an onboard radioactive calibration source, most of the remaining lines can be understood in terms of cosmic-ray induced nuclear transitions in the telescope structure and have been seen by other investigators with comparable intensity and width[3,4].

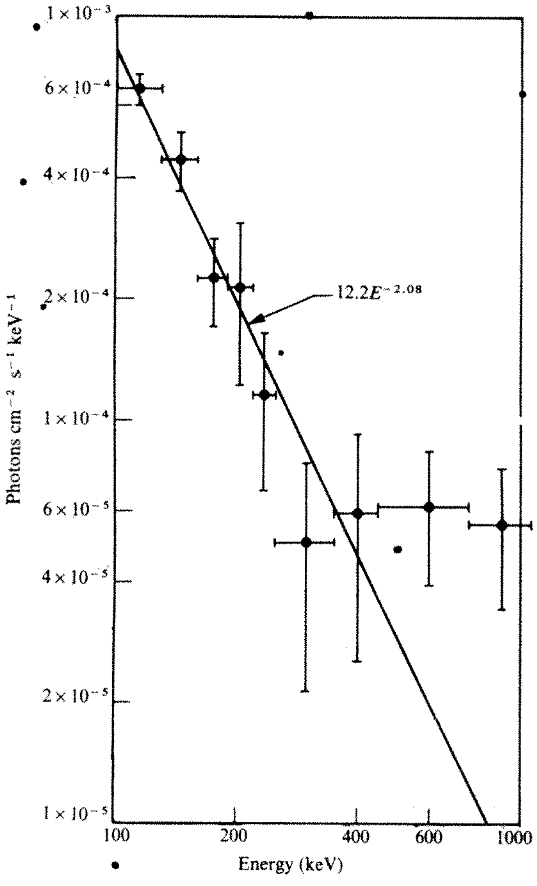

Fig. 2 Energy spectrum of the Crab Nebula's total emission at the top of the atmosphere. The solid line is the power law that best fits the experimental data. The data have been collected into 30-, 100- and 300-keV bins to reduce the statistical scatter of the points.

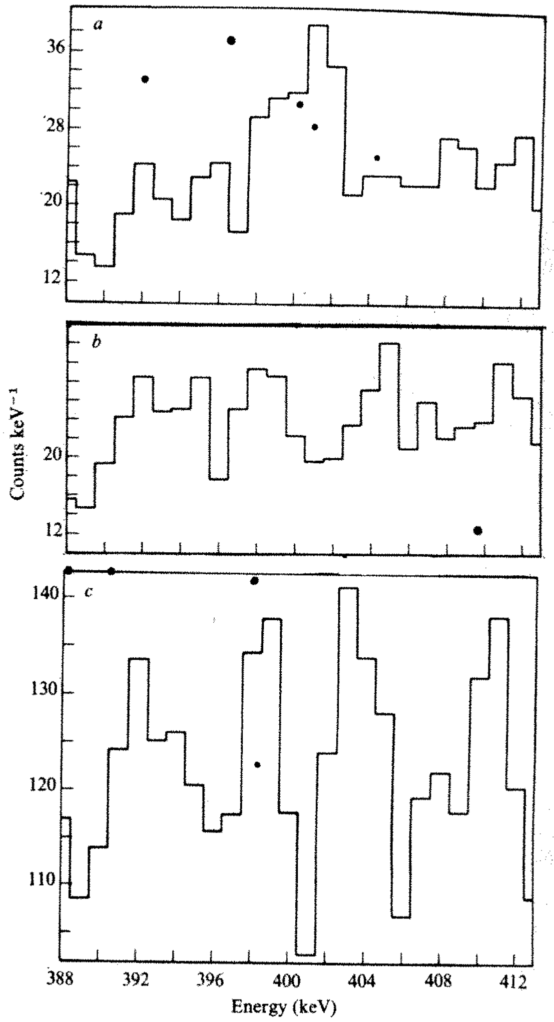

Fig. 3 *a*, Crab target and *b*, Crab background energy spectra in the vicinity of 400 keV; *c*, a composite spectrum of all data less Crab target segments.

In addition to the lines mentioned above, one potential line feature has been detected at 400±1 keV in the target data but not in the background data. Both the Crab target and Crab background spectra in the vicinity of 400 keV are shown in Fig. 3, with a spectrum consisting of a sum of all our data excluding the Crab target segments. These spectra are basically raw data, having been corrected only for drifts in the energy calibration with time. It is believed that the telescope continuum at this energy is dominated by leakage of the atmospheric γ-ray background through the NaI shield. The fact that the feature appears only in the Crab target spectrum is taken as strong evidence that it is not a telescope artefact. It is also important that the line is evident in most of the target segments. The feature represents a 4.0σ deviation above the telescope continuum as determined from the 387.5–397.5 and 402.5–412.5 keV energy bins of the target spectrum. Although the above method was originally used, there are many other valid ways to search the data for lines, each yielding a slightly different level of significance for the 400-keV feature, for example, one can vary the width of the relevant energy bins, theoretically extrapolate the data to the top of atmosphere before searching for lines, use the Crab background data for continuum subtraction, etc. Using these methods, the level of statistical significance can be made to vary from ~ 3.3σ to ~ 4.2σ. Clearly, the probability that the feature is a statistical accident is not negligible, and definitive proof of its reality must await further experimentation. After correcting for atmospheric attenuation and dead time, the feature in Fig. 3 corresponds to a line flux of $2.24 \pm 0.65 \times 10^{-3}$ photons s^{-1} cm^{-2} incident upon the Earth with a width $\lesssim 3$ keV. Such a line flux could have produced a feeble feature in the previously most sensitive search for lines from the Crab Nebula[1] made with an instrument of much poorer energy resolution. The absence of a strong indication of it in these earlier data signals a note of caution in accepting the reality of the present result.

Finally, it is interesting to consider a possible physical origin for the feature. Several mechanisms exist for generating positrons at or above the surface of the Crab neutron star[5,6]. Positron annihilation in dense gases and solids usually results in the emission of 511 keV photons. Should this occur near the stellar surface, the photons would appear to us gravitationally redshifted. This measurement then determines $Z_{\mathrm{Crab}} = 0.28$ (assuming no other sources of frequency shift). Recent neutron star models[5,7] can then be used to determine the mass of the Crab yielding ~ 1.4 $M_{\odot}$ which is not unreasonable[7]. For this hypothesis to be correct, ~ 10^{41} positrons s^{-1} must be annihilating into 511 keV photons near the neutron star surface (assuming isotropic radiation) which then reach Earth without significant Compton scattering. A more detailed report of the experiment will be published elsewhere.

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Interpretation of observed cosmic microwave background radiation

THE observed cosmic microwave background radiation, which has a high degree of spatial isotropy ($\Delta T/T \leqslant 10^{-3}$) and which closely fits a 2.7 K black body spectrum, is generally claimed to be the strongest piece of evidence in support of hot big bang cosmologies by its proponents (for a recent review see ref. 1). Alternative explanations in terms of the integrated effect of a suitable population of extragalactic radio sources[2–6] have been criticised, essentially on the ground that there is no known population of extragalactic objects with a source density sufficient to explain the observed small-scale isotropy of the microwave background[7]. We report here that the 'surface of last-scattering' of the observed microwave background radiation corresponds to the distribution of dust in galaxies or protogalaxies with a temperature ≈ 110 K at the epoch corresponding to $Z \sim 40$, and not to a plasma of temperature $\gtrsim 3{,}000$ K at an earlier epoch ($Z \gtrsim 1{,}000$), as given by the canonical model of big bang cosmologies. The claim that this radiation lends strong support to hot big bang cosmologies is without foundation.

Wickramasinghe *et al.*[8] assumed the existence of dust uniformly distributed in intergalactic space. Absorption and re-emission of radiation from galaxies by these grains then produce the microwave background radiation. This dust must have special geometrical properties to make the metagalaxy up to the Hubble radius optically thick around $\lambda \leqslant 2{,}000$ μm, but allowing it to be transparent in the visual range. Based on radar scattering data for thin wires, semiconducting needles (for instance, graphite) with length $l \approx 100$–$1{,}000$ μm and cross-sectional radius $a \ll l$ are expected to have this effect, as they seem to produce a strong peak in the absorption cross-section around $\lambda \approx 2l$. Also, the required intergalactic dust density of 10^{-34} g cm^{-3} is not incompatible with upper limits set by other methods[9,10].

The observation of very distant discrete radio sources down to mm wavelengths presents a serious difficulty for this model. The QSOs with the two largest red shifts (OH 471 at $Z = 3.40$ and OQ 172 at $Z = 3.53$) are at distances from us of 0.90 R_0 and 0.91 R_0 respectively (where R_0 is the present Hubble radius). Yet they both indicate a second maximum in their emission spectrum[11] at $\lambda \approx 3{,}000$ μm, where the above model implies an optical depth $\gtrsim 4.5$.

We therefore conclude that the microwave background radiation must be explained not in terms of a coupling between matter and this radiation at the present epoch, but in terms of a coupling in a previous epoch within the framework of an evolutionary cosmology.

Rather than specifying a certain evolutionary cosmology (that is, the 'initial conditions'), let us merely go back in time from the present epoch. If the coupling between the radiation and matter occurred when the radius of the metagalaxy was R, then the common thermal equilibrium temperature of matter and radiation is given by

$$T_B \approx 2.7\,(R_0/R)\ \mathrm{K}, \qquad (1)$$

while the wavelength of maximum radiation is given by

$$\lambda_m \approx 1{,}800\,(R/R_0)\ \mu\mathrm{m}. \qquad (2)$$

Provided the total number of absorbers of the radiation at any given wavelength λ is conserved, the optical depth of the metagalaxy at λ is given

$$\delta(\lambda) = \delta_0(\lambda)\,(R_0/R)^2 \qquad (3)$$

where δ_0 is the optical depth at present. If the absorbers are uniformly distributed in the metagalaxy with a present number density n_0 and cross-section $\alpha(\lambda)$, then

$$\delta_0(\lambda) = \alpha(\lambda)\, n_0 R_0. \qquad (4a)$$

If, however, the absorbers are concentrated in galaxies then

$$\delta_0(\lambda) \approx \delta_g(\lambda)\, N_{0g} S_g R_0, \qquad (4b)$$

where $\delta_g(\lambda)$ and S_g are respectively the average optical depth of a galaxy at λ and its average geometrical cross-section (which are assumed to remain unchanged), and N_{0g} is the present number density of galaxies in the metagalaxy.

If the absorbers are uniformly distributed in intergalactic space, one can easily make the metagalaxy optically thick[8] at epochs given by $R/R_0 \leqslant 0.1$ with a present dust density $\leqslant 10^{-36}$ g cm^{-3}, without conflicting with the above observations.

Instead of postulating the existence of an unobserved population of intergalactic dust, however, we will work in terms of the known properties of dust distribution in our own Galaxy. The sources of absorption and re-emission of the radiation in past epochs are then taken to be the dust distributions in dusty spiral galaxies, which we take to be similar to that of ours. The condition needed for thermalisation of the background radiation at any epoch is given by $\delta(\lambda_m) \approx 1$.

We do not know the extinction in our own Galaxy at far infrared wavelengths, but the visual extinction $A_g(\lambda_v)$ parallel to the galactic pole[12] for its entire thickness ≈ 0.5 m. As long as the grains have a sufficient conductivity (for example, graphite, 'dirty' ice, etc.) their extinction is expected to follow approximately an inverse wavelength dependence for $\lambda \gg 2\pi a$ (where a is the grain radius)[13,14] and there is some supporting observational evidence[15] in the range $50\ \mu\text{m} \leqslant \lambda \leqslant 1{,}000\ \mu\text{m}$. Assuming this to be the case, and taking $\lambda_v \approx 0.55\ \mu\text{m}$ we get $A_g(\lambda_m) \approx 0.5 \times 0.55\ \lambda_m^{-1}$ and a corresponding optical depth $\delta_g(\lambda_m) \approx 0.92 A_g(\lambda_m) \approx 0.25 \lambda_m^{-1}$, which is the optical depth appropriate to our calculation, as the dust is believed to be concentrated in a thin disk of thickness $\leqslant 200$ pc (ref. 16).

The total population of galaxies in the metagalaxy is also highly uncertain and depends on the highly uncertain value of the deceleration parameter q_0. Taking the current 'best estimate'[17] of q_0 to be 0.03 and assuming a negligible mass of intergalactic matter, we estimate a metagalactic population of around 2×10^{10} dusty spiral galaxies, giving $N_{og} \approx 1.2 \times 10^{-75}$. Then with $R_0 \approx 1.6 \times 10^{28}$ and $S_g \approx 5 \times 10^{45}\ \text{cm}^2$ we get $N_{og} A_g R_0 \approx 0.1$. Substituting these values in (3) and using (2) we get

$$\delta(\lambda_m) \approx 1.4 \times 10^{-5} (R_0/R)^3 \qquad (5)$$

whence $\delta(\lambda_m) \approx 1$ when $R_0/R \approx 41$. We conclude therefore that the background radiation was coupled to the galactic dust at this epoch $Z = (R_0/R) - 1 \approx 40$, when they both had the common equilibrium temperature ≈ 110 K. There is no difficulty for any type of hitherto proposed interstellar grain, including H_2O ice, to exist at this temperature.

The epoch of galaxy formation is highly model dependent and uncertain, as is the epoch of dust production. The current best estimates[18] of the epoch of protogalaxy formation in an expanding universe are $Z = 10$, 20 and 60 for $q_0 = 2$, 0.5 and 0.02, respectively, and do not apply to a universe which had a bounce in the past. Considering this and the order of magnitude nature of our own calculation, our implicit assumption that galaxies (or at least dusty protogalaxies) existed at $Z \approx 40$ is not unreasonable. Also, at $Z \approx 40$, the galaxies still occupy $\leqslant 1\%$ of the volume of the metagalaxy.

The number density of galaxies (or protogalaxies) at this epoch ($Z \approx 40$) $\gtrsim 7 \times 10^4\ N_{og}$, and this easily exceeds all lower limits for this number density of discrete sources set by the observed small-scale spatial isotropy of the microwave background radiation[7].

Just as the radiation received from the Sun gives us direct information only about its surface, the microwave background radiation gives us direct information only about the 'surface of last scattering' when the radiation finally decoupled from matter and beyond which the metagalaxy remained transparent. According to our model, this 'surface of last scattering' was the total dust distribution in galaxies or protogalaxies at the epoch $Z \approx 40$. Consequently, we cannot obtain direct information about the metagalaxy at earlier epochs ($Z > 40$), as it was then opaque. This conflicts with the detailed canonical model of the hot big bang cosmology which identifies this 'surface of last scattering' with a rapidly recombining plasma of temperature $T \approx 3{,}000$ K at a much later epoch $Z \gtrsim 1{,}000$, when the coupling mechanism between the matter and radiation was Thompson scattering (for example, see ref. 19).

Despite this conflict, however, we do not claim that the observed microwave background radiation is inconsistent either with the basic concept of a hot big bang cosmology, or with a completely different class of evolutionary cosmology, for example, the symmetric cosmology of Klein[20-22], where the minimum radius of the metagalaxy was $> 0.01\ R_0$.

We stress that the observed cosmic microwave background radiation does not give us direct information about the state of the metagalaxy further back than the epoch corresponding to $Z \approx 40$, when galaxies or protogalaxies already existed, and when its own temperature was only about 110 K. To claim that it is strong evidence in support of the hot big bang cosmology whose earliest epochs correspond to $Z \gtrsim 10^{12}$ with corresponding temperatures of matter and radiation $> 10^{10}$ K, is completely unjustified.

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Radiative acceleration of gas above a luminous disk

Spherically symmetric radiative acceleration has been considered[1] as a mechanism for the ejection of matter from quasistellar objects. I present some calculations showing that in a simplified case, a strongly beamed outflow will result if the source of the driving radiation is not point-like but a disk.

Consider an infinitesimally thin, optically thick disk that emits thermal radiation with an intensity $I(r)$. The radiative force exerted by a surface element $r\,\mathrm{d}r\,\mathrm{d}\varphi$, situated at cylindrical coordinates $(r, \varphi, 0)$, on a spherical cloud with cross section S is

$$\mathrm{d}F = (S/c)\ [2hr\,I(r)(h^2 + R^2 + r^2 - 2rR\cos\varphi)^{-3/2}] \qquad (1)$$

$$\times (h^2 + (R - r\cos\varphi)^2)^{-\frac{1}{2}}\,(h, R - r\cos\varphi)\,\mathrm{d}r\,\mathrm{d}\varphi$$

where $(R, 0, h)$ are the cylindrical coordinates of the cloud. If the distance $D \equiv (h^2 + R^2)^{\frac{1}{2}}$ of the cloud from the disk centre is large compared with the effective disk radius, one finds by direct integration of equation (1)

$$F \simeq \frac{SL}{2\pi c}\,\frac{h}{D^3}\,\boldsymbol{e} \qquad (2)$$

where L is the total luminosity of the disk and $\boldsymbol{e}$ is a unit vector pointing radially away from the disk centre. The net force on a cloud with mass m is due to the sum of F and the gravitational attraction of the disk and its central object. If the latter is dominant and has mass M, the net force is

$$F = \frac{\boldsymbol{e}}{D^2}\left(\frac{SL}{2\pi c}\,\frac{h}{D} - MGm\right) \qquad (3)$$

Apparently, this force is directed away from the central object if

$$h \gtrsim R(4\varepsilon^2 - 1)^{-\frac{1}{2}},\ \varepsilon \equiv SL/(4\,\pi\,mc\,MG) \qquad (4)$$

where ε is the Eddington number. Equation (4) defines a cone above the disk, the opening angle of which becomes larger with

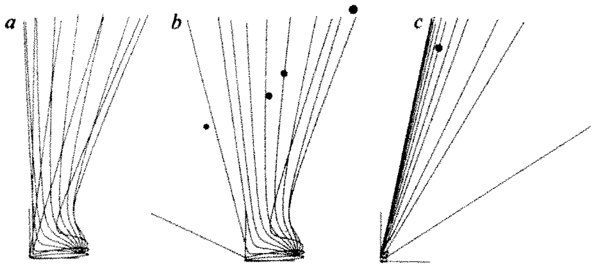

Fig. 1 Orbits of clouds in the force field given by equation (3), with Eddington number $\varepsilon = 1$. Solutions were made dimensionless by scaling with length unit r_0 (horizontal and vertical lines) and time unit $(MGr_0^{-3})^{-1/2}$. Plot marks indicate time intervals of 0.5. *a*, All initial velocities in the plane of the drawing. Initial velocity modulus 0.5, stepped by 15° from −90° to +90°. *b*, Initial velocities as in (*a*), but all with initial velocity 0.1 added tangential to the disk. *c*, Same rotated through 90°. Every cloud passing through the disk is probably stopped there, which makes the beam narrower than shown here.

increasing luminosity L. The existence of this cone is the cause of the beamed outflow. Equation (4) has solutions if

$$\frac{2\pi m}{S} < \frac{L}{MGc} = 9.62 \times 10^{-3} \left(\frac{LM_{\odot}}{L_{\odot}M} \right) \text{kg m}^{-2} \qquad (5)$$

The cloud must be optically thick, so that $m/S > m_{\mathrm{H}}/\sigma$ where σ is a scattering cross section. If σ equals the Thomson cross section, the 'cloud' is an individual particle, and equation (5) is the Eddington condition.

I have considered two types of motion in the far field (3), namely the motion of individual clouds or particles, and the motion of an atmosphere. The former is a straightforward problem of classical mechanics, which was solved numerically by a fourth order predictor–corrector method (Fig. 1). The motion of a stationary atmosphere was considered for a dust model (negligible gas pressure). In spherical coordinates (r, φ, θ) the equations for this case are

$$uu_r + \frac{v}{r} u_\theta - \frac{v^2}{r} = \frac{MG}{r^2} (2\varepsilon \cos\theta - 1) \qquad (6)$$

$$uv_r + \frac{v}{r} v_\theta + \frac{uv}{r} = 0 \qquad (7)$$

$$\rho\left(u_r + 2\frac{u}{r} + \frac{1}{r} v_\theta + \frac{v}{r} \cot\theta\right) = -u\rho_r - \frac{v}{r} \rho_\theta \qquad (8)$$

where $(u, 0, v)$ is the velocity vector and a subscript denotes partial derivation. The full equations, including azimuthal motion and a viscous dissipation term, also admit the type of similarity solutions that will be described below. These have been calculated also, but are omitted here for the sake of brevity; they are alike to the solutions given here, so that the main conclusions regarding the beaming and the influence of the Eddington number are not altered. Separating the variables by putting $v = f(\theta)\,(MG/r)^{1/2}$ and $u = -2\,(MG/r)^{1/2}\,\partial f(\theta)/\partial\theta$, one obtains the ordinary differential equation

$$2\left(\frac{\mathrm{d}f}{\mathrm{d}\theta}\right)^2 + 2f\frac{d^2f}{\mathrm{d}\theta^2} + f^2 = -2\,\varepsilon\cos\theta + 1 \qquad (9)$$

The streamlines follows from u and v as

$$\frac{u}{v} = \frac{1}{r}\,\frac{\mathrm{d}r}{\mathrm{d}\theta} = -2\,\frac{\mathrm{d}\log f}{\mathrm{d}\theta} \rightarrow r \propto f^{-2} \qquad (10)$$

The density is found from equation (8) to be

$$\rho = \psi\, r^A f(\theta)^{2+2A}/\sin\theta \qquad (11)$$

Further solutions for ρ can be generated from equation (11) by putting $\psi = \psi(A)$, solving A from $(\partial\rho/\partial A) = 0$, and substituting the solution into (11). Equation (9) has the solution

$$f = \{1 - 2\,\varepsilon[\alpha\cos\theta + (\beta + \tfrac{1}{2}\,\theta)\sin\theta]\}^{1/2} \qquad (12)$$

The boundary conditions at $\theta = \pi/2$, $r = \infty$, dictate $\beta = 1/(2\varepsilon) - \pi/4$ and $\alpha = \frac{1}{2} - \mu/\varepsilon$, where μ is a small number determining the thickness $z = r_c f^2(\theta_c)/2\mu$ of the disk at infinity. Here θ_c is the angle at which the flow is just tangential to the central object with radius r_c (Fig. 2). The boundary conditions at $r = r_c$ determine the value of ε implicitly, as follows. Integration of ρu over the region $\theta_c < \theta < \pi/2$ gives the accretion rate

$$\dot{M} = 4\pi\psi\, r_c^{A+2} f_c^{2A+3} (MG/r_c)^{1/2}/(2A+3) \qquad (13)$$

where $f_c = f(\theta_c)$. Because $L \approx 3M\dot{M}G/r_c$, it follows from equation (4) that

$$\gamma\varepsilon = f_c^{2A+3},\ \gamma = \frac{mc}{S}\,\frac{2A+3}{3\psi r_c^A}\,(MGr_c)^{-1/2} \qquad (14)$$

Now θ_c is the solution of $(\partial f/\partial\theta)(\theta_c) = 0$; because $f = f(\varepsilon, \mu)$ one has $\theta_c = \theta_c(\varepsilon, \mu)$, whence equation (14) becomes an implicit equation for ε with solution $\varepsilon(\mu)$. For given boundary conditions at $r = \infty$, there is only one ε that makes the flow fit at $r = r_c$ (Fig. 2).

To obtain the force field near the disk, equation (1) must be integrated numerically. This precludes simple analytic hydrodynamics, so I have only numerically integrated the equations of motion for individual clouds or particles in the near field (Fig. 3).

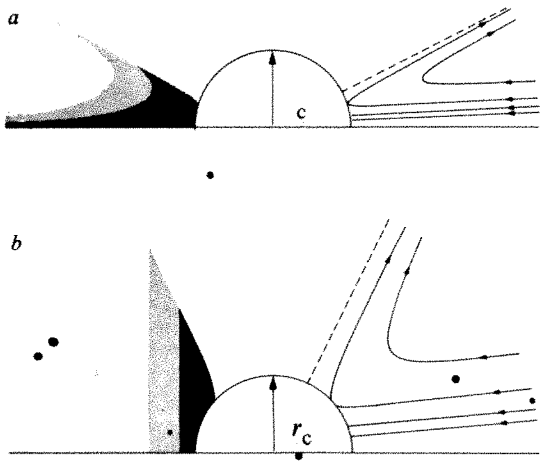

Fig. 2 Gas flow in the far field of the disk, if the gas pressure is negligible. The solutions have been made self-consistent by requiring that the accretion onto the central object with radius r_c generates exactly the luminosity corresponding to the Eddington number of the flow pattern. *b*, $A = -1$ [see equation (1)], $\mu = 0.01$; these parameters imply $\varepsilon = 1.42$ and a disk thickness at infinity of $z = 4.74\ r_c$. *a*, $A = -1.25$, $\mu = 0.01$, yielding $\varepsilon = 3.43$ and $z = 0.98\ r_c$. It has been assumed that σ equals the Thomson cross section, $\psi r_c^A = 10^{-2}$ kg m^{-3}, $r_c = 10^6\ m$, $M = M_{\odot}$, and m equals the mass of the proton. It seems that for $0 < \mu < 1$, the self-consistent Eddington number is $1.35 < \varepsilon < 3.57$ if $A = -1$, and $3.25 < \varepsilon < 6.83$ if $A = -1.25$. Further exploration of equation (14) indicates that for reasonable γ, ε is typically rather less than 5.

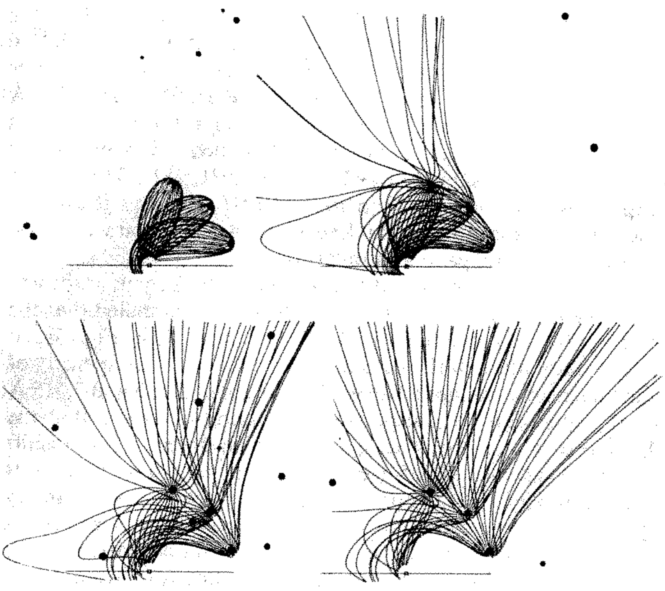

Fig. 3 Orbits of particles in the near force field of equation (1). The particles were radiated isotropically in the plane of the drawing with initial velocity 0.25 (normalisation as in Fig. 1), starting one disk radius away from the central object. Eddington numbers are 2, 3, 4, and 5, respectively. Note that even near the disk, beaming is rapidly established. The % of particles blown away is, respectively: 0, 18, 47, 70. These high values suggest a self-regulatory mechanism for the disk luminosity, by non-acceptance of infalling material when $\varepsilon \gtrsim 2.5$. Since strong beaming occurs for low ε only, this regulation indirectly helps to maintain the collimated outflow.

Apparently it is possible to establish strong beaming within a distance of the order of the disk radius. Thus X-ray sources with double radio structure[2,3] and extragalactic radio doubles could in principle derive their shape from an asymmetry of their central radiation field. A proper treatment of this asymmetric acceleration, however, will have to deal with radiative transfer[1] and must include considerations about the spectrum of the disk radiation, Rayleigh-Taylor instability of the accelerated material (which perhaps gives rise to clouds in the first place), and possibly the influence of magnetic fields. The problems involved are too extensive to be readily estimated, but probably the beaming will persist even when some radiation scattering is present. The reason to expect this is that, to first order, the radiation removed from the anisotropic component of F by scattering through an optical thickness τ will emerge as isotropic radiation pressure, which counteracts the influence of gravity:

$$Fr^2/MG \approx 2\varepsilon e^{-\tau}\cos\theta + \varepsilon(1-e^{-\tau})-1 \qquad (15)$$

Near $\varepsilon = 1$, the value of θ where $F = 0$ (that is, the opening angle of the cone within which the force is directed outward) does not depend strongly on τ; for example if $\varepsilon = 1.5$, the opening angle increases from 70° at $\tau = 0$ to 90° at $\tau = 1.1$.

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Magnetic field amplification in interstellar collisionless shock waves

IT is commonly assumed that a simple compression of the magnetic field occurs in interstellar shock waves. Recent space observations of the Earth's bow shock show that turbulent amplification of the magnetic field can occur in a collisionless shock. I show here that radio observations of Tycho's supernova remnant indicate the presence of a shock wave with such magnetic field amplification.

A collisionless shock wave can be characterised by three parameters: the Mach number, M, or the Alfven Mach number, M_A; the preshock ratio of thermal to magnetic pressure, β; and the angle between the flow direction and the magnetic field, θ (compare with ref. 1). Formisano and Hedgecock[2] classified the structures of the Earth's bow shock as a function of these parameters. They found that in shocks with $M_A > 3$, the degree of turbulence increased with β. The dividing line between laminar and turbulent flow occurred at $\beta \sim 0.1$. A series of observations of high β bow shock structures have been reported by Formisano *et al.*[3], who found magnetic field increases in the shocks which were much larger than normal. Typical field increases are by a factor of 2–4, but here increases by a factor of 20 or more were noted. In fact, in the first shock ($\beta = 8.0$, $M = 6.5$), the energy density in the postshock field was about equal to the dynamic pressure of the upstream solar wind; in the second ($\beta = 170$, $M = 7.4$), it was $\sim 15\%$ of the wind pressure, and in the third ($\beta = 49$, $M = 7.3$), it was $\sim 30\%$ of the wind pressure. There is at present no theory for the macroinstabilities which give rise to the turbulent amplification of the magnetic field.

Despite the lack of theoretical understanding, the possibility of field amplification in interstellar shock waves should be considered. In Tycho's supernova remnant, there is evidence for the presence of a collisionless shock. M and β of a shock are

$$M = 7.1\,(V_s/100\ \mathrm{km\ s^{-1}})\,(T/10^4\ \mathrm{K})^{1/2}$$

$$\beta = 3.9\,(T/10^4\ \mathrm{K})\,(n/1\ \mathrm{cm^{-3}})\,(B/3\times10^{-6}\ \mathrm{G})^{-2}$$

where V_s is the shock velocity and T, n, and B refer to the temperature, density, and magnetic field of the ambient medium which is assumed to be ionised. M_A is proportional to $M\beta^{1/2}$. The distance to Tycho's remnant is uncertain; distance dependent quantities will be be given in terms of D_6, which is in units of 6 kpc (refs 4, 5). Motions of the optical filaments in Tycho's remnant imply a velocity of 6200 D_6 km s^{-1} (ref. 6). The filaments appear along the outer edge of the radio emission (J. R. Dickel, personal communication), so they probably outline the shock front. In many places, the outer edge of the radio emission is unresolved at 6 arc s resolution[7], again providing evidence that this defines the shock front. The Coulomb mean free path of the hot postshock gas is a significant fraction of the radius of the remnant, indicating that collisionless heating of the gas occurs. The Mach number is larger than 100, which is greater than the Mach number of observed shock waves in the Solar System. There is no reason to expect a collisionless shock to become less turbulent with increasing M.

The height of Tycho's remnant above the galactic plane is 160 D_6 pc. The density and magnetic field are not well known at this altitude, but it is likely that $\beta > 1$. X-ray observations of the remnant indicate a high ambient density, $n \sim 4\ \mathrm{cm^{-3}}$ (ref. 8). Stability of the gas to the Rayleigh–Taylor instability in the galactic gravitational field may also require $\beta > 1$. Thus, on the basis of observations of the Earth's bow shock, it is expected that turbulent magnetic field amplification occurs in the shock wave of Tycho's remnant.

This hypothesis is supported by the radio observations of the remnant. Van den Bergh[6] found evidence from the filament motions that the remnant is in the adiabatic blast wave phase of evolution. The postshock flow can be described by a similarity solution. Duin and Strom[7] found that the observed radio polarisation, which implies a radial orientation of the magnetic field, could be quantitatively accounted for by the similarity solution because of the radial stretching in the postshock flow. This model is, however, viable only if the magnetic field is randomised in the shock wave. If only tangential compression occurred in the shock, a very particular magnetic field alignment would be necessary to give the observed polarisation direction. Because a radial field is implied by observations of several young remnants, this is unlikely. Thus, we have evidence that the magnetic field has been randomised in a turbulent collisionless shock.

Next, we consider to what extent the magnetic field is amplified in the shock wave on the basis of the nonthermal radio flux. First assume that there is no turbulent field amplification. The postshock magnetic field is probably less than 1.2×10^{-5} G because the ambient field is likely to be smaller than 3×10^{-6} G. If this field occupies 25% of the supernova remnant volume, the magnetic energy is $5\times10^{46}\,D_6{}^3$ erg. In order to produce the observed nonthermal radio radiation, a relativistic electron energy of $2\times10^{49}\,D_6{}^2$ erg is required. This is an extreme lower limit to the relativistic particle energy because it assumes that the magnetic field 160 D_6 pc from the galactic plane is the same as that in the plane, that the maximum field compression occurs in the shock, and that there is negligible energy in relativistic protons; the implied energy may actually be an order of magnitude higher. As the total energy release in the supernova is probably less than 10^{51} erg, this is a large fraction of the supernova energy. It is unlikely that such a high acceleration efficiency can be achieved, especially because adiabatic deceleration would be the main process acting on the particles in the absence of turbulence. On the assumption that there is no energy in relativistic protons, the equipartition field in Tycho's remnant is $6\times10^{-5}\,D_6{}^{-2/7}$ G, which would be accompanied by a particle energy of $2\times10^{48}\,D_6{}^{17/7}$ erg. The corresponding magnetic field pressure is only $0.02n^{-1}\,D_6{}^{-18/7}$ % of the dynamic shock wave pressure. The required increase in the magnetic field, a factor of about 20, is comparable to that observed in high-β bow shocks. It is likely that the magnetic field has been turbulently amplified in the collisionless shock wave.

The turbulent hydromagnetic motions may result in stochastic particle acceleration. Energetic particles are frequently observed in association with collisionless shocks in the Solar System. This subject is important in considering the radio emission from supernova remnants in the adiabatic phase of their evolution. The possible presence of turbulent collisionless shocks should also be considered in models of active galactic nuclei and double lobe radio sources.

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Lack of dust in quasar absorption line systems

THE origin of the absorption line systems in quasars is still uncertain[1]. Most such systems apparently have column densities of atomic hydrogen of the order of 10^{19} cm^{-2}, but, at least two quasars, 1331+170 (ref. 2) and PHL 957 (ref. 3), have such strong Lyman α absorption lines that atomic hydrogen column densities of the order of 10^{21} cm^{-2} are indicated. It should be possible to observe the dust produced 2,200 Å extinction bump as it is redshifted into the visible[4], and to determine whether the absorption line systems are produced in spiral galaxies where the dust content is similar to that in the interstellar medium. McKee and Petrosian[4] argue that the emission line regions of quasars generally lack dust, and that toward PHL 957 the 2,200 Å feature is absent. From recent published data, I argue that dust similar to that found in the interstellar medium is not found toward the quasars 1331+170 and PHL 957. This could explain why H_2 is not found toward PHL 957, and it also indicates that the absorption line systems in quasars are not produced in spiral galaxies similar to ours.

Carswell *et al.*[2] measured the profile of the Lyman α absorption line toward 1331+170, from which they estimated that the line centre is redshifted to 3,370 Å. From their Fig. 3*a*, it seems that although the data are noisy, the residual intensity of the line profile reaches a value of 0.5 at about 3,335 and 3,415 Å to the blue and red, respectively. It therefore seems that the Lyman α line centre is at 3,375 Å, which agrees with redshift system A at $z = 1.7755$ (refs 2, 5). There is also a redshift system at $z = 1.7852$, but in general the equivalent widths in system B are smaller than the equivalent widths in system A (ref. 2). It is likely that most of the hydrogen toward 1331+170 is in system A. In any case, the Lyman α line is so wide that it probably lies on the damping portion of the curve of growth, and the precise line centre does not affect the estimate of the atomic hydrogen column density, N(H). (Observations with higher signal-to-noise should be obtained to be certain that the Lyman α profile can be represented by damping wings). The Lyman α half width at half maximum (HWHM) is 40 Å. In the rest frame of the absorption line system, the HWHM is 14 Å, and N(H) equals 3×10^{21} cm^{-2} (ref. 6).

In our Galaxy, the dust-to-gas ratio is generally uniform to within a factor of two (ref. 7). We adopt $E(B\text{-}V)/N(\mathrm{H}) = 0.2\times10^{-21}$ mag cm^{-2} (refs 8, 9). Assuming a ratio of total to selective extinction of $R = 3.3$ (ref. 10) and taking the standard extinction curve for the 2,200 Å bump of Bless and Savage[11], then for representative wavelengths the dust optical depth may be written as $\tau(2{,}500) = 6.8\,E(B\text{-}V)$, $\tau(2{,}170) = 9.3E(B\text{-}V)$ and $\tau(2{,}000) = 8.2\,E(B\text{-}V)$. In all stars with sufficiently great reddening for the extinction to be measured accurately, this bump is present with a strength within a factor of two of its average values[12].

If $N(\mathrm{H}) = 3\times10^{21}$ cm^{-2} toward 1331+170 and if the dust-to-gas ratio in the absorption line system is similar to that in the interstellar medium, we would expect that $E(B\text{-}V) = 0.6$, and would expect to observe dust optical depths at 6,940, 6,020 and 5,550 Å of 4.1, 5.6 and 4.9, respectively. If lines are ignored, the flux density between 5,550 and 6,940 Å is observed to be constant to within a factor of 1.2 (ref. 5). If the dust-to-gas ratio were normal and assuming a flat spectrum, we would expect flux densities at 6,020, 5,550 and 6,940 Å to be in the ratios 1:2:4. Although a more precise analysis is possible, it seems that the dust-to-gas ratio toward 1331+170 is at least a factor of 20 less than in the interstellar medium.

From the measured equivalent widths[2] of the Fe II and Si II lines in the $z = 1.7755$ system, we find that even if these lines lie on the linear portion of the curve of growth, these species have column densities on the order of 3×10^{14} cm^{-2}. Therefore the gas phase abundances of the refractory elements iron and silicon toward 1331+170 is comparable to the gas phase abundances of the same species toward ζ Oph (ref. 13). There is no reason to suppose that the lack of dust toward 1331+170 results from a lack of metals.

The column density of atomic hydrogen toward PHL 957 is $7.5\pm2.5\times10^{20}$ cm^{-2} (ref. 14). Except for a very broad C III line, between 6,000 and 9,000 Å the flux density seems to be a power law within a factor of 1.2 (ref. 14). In the absorption line system at $z = 2.3088$, we would expect a flux density variation between 7,180 and 8,270 Å (corresponding to 2,170 and 2,500 Å in the rest frame) of a factor of about 1.5. It therefore seems that there is a lack of normal dust toward PHL 957 by at least a factor of two, in rough agreement with McKee and Petrosian's estimate[4].

I conclude that the absorption line region toward 1331+170 and probably the region toward PHL 957 are lacking dust similar to that in our own Galaxy. This can explain the lack of H_2 in these systems[14,15]. The column density of atomic hydrogen toward 1331+170 is so high, however, that it is unlikely that we are observing this quasar through a galactic halo[16] where the dust content might be unusually low. In the disk of a spiral galaxy, we would expect the dust/gas ratio to be comparable to the value in the interstellar medium, and it seems unlike that the absorption line system at $z = 1.7755$ toward 1331+170 is produced in a spiral galaxy similar to ours.

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Relationship between atmospheric electricity and microwave radio propagation

ABRUPT changes in received polarisation coincident with lightning strokes have been seen on a 20 GHz satellite-to-earth radio link. These effects, and the surprisingly high levels of atmospheric cross polarisation observed on satellite links, have been tentatively attributed to high altitude ice crystals and their possible alignment by electric fields[1,2]. In this paper we shall add to the existing data by presenting a precise measurement of the time scale of the abrupt polarisation changes and an event showing a striking correlation between the electric potential gradient at the ground and the cross-polarisation of signals received from the satellite. This is believed to be the first time that direct measurements of atmospheric electric fields have been related to microwave propagation measurements.

The linearly polarised 20 GHz beacon signal from the NASA ATS-6 geostationary satellite was received at the Bradford University Oxenhope Laboratory during the period October 1975 to July 1976, while the satellite was stationed at longitude 35°E. At the receiving site (which is 450 m above sea level) the satellite was visible at 20° elevation with a 23° polarisation counter-clockwise from the vertical. Both the 'copolarised' and the orthogonal (or 'cross-polarised') received signals were measured. Without atmospheric hydrometeors present on the radio path, the cross-polarised component was typically >35 dB below the co-polarised component. A co-sited range-gated radar provided a quantitative measurement of reflectivity along the satellite radio path[1].

It is now apparent that the cross-polarisation measured on a microwave or millimetre wave satellite radio path can be an order of magnitude greater than that experienced on a terrestrial link for the same attenuation[1–3]. The cross polarisation measured on a terrestrial path agrees with that predicted from a rainfall model assuming anisotropy to arise from non-spherical raindrops[3,4], whereas small, high altitude ice particles (possibly needles or plates) can cause significant cross-polarisation on a satellite path[1,2,5,6]. This high-cross polarisation, not related to attenuation, as observed on satellite radio paths has been termed 'anomalous' although this description may be questioned.

During 1,050 h of measurements with ATS-6 (in discrete sessions of typically 4 h per d) nine separate 'anomalous' events were recorded (that is, events involving separate weather structures). Typically, as a rainstorm moved over the receiving site, high (anomalous) cross-polarisation, varying on a time scale of minutes, was observed at such times when the slant radio path intercepted the frozen particles in the storm structure[1]. In addition, during a thunderstorm on 7 May, 1976, abrupt changes in cross polarisation were observed (Fig. 1), in which the cross-polarised signal increased suddenly by several dB relative to the copolar level, and then returned close to the value before discharge in ~45 s. Of the twenty abrupt changes recorded during this event, four coincided with lightning strokes, as noted by observers on site for a short period of the storm. The other abrupt changes in cross-polarisation may also have been associated with lightning.

The rise times of the 15 largest abrupt cross-polarisation changes were found to be between 0.1 and 0.25 s, from FM tape recordings taken in a 500 Hz bandwidth. Sixteen of the abrupt changes showed a rapid rise in cross-polarisation, whereas the remaining four were decreases. These changes are self evidently not 'sferics' (in view of the relatively long decay times) and were only seen in the one event during ATS-6 sessions which showed pronounced lightning activity. Identical changes were observed with two independent, coherent receivers for the cross-polarised signals and furthermore, no abrupt changes were seen in the co-polarised signal.

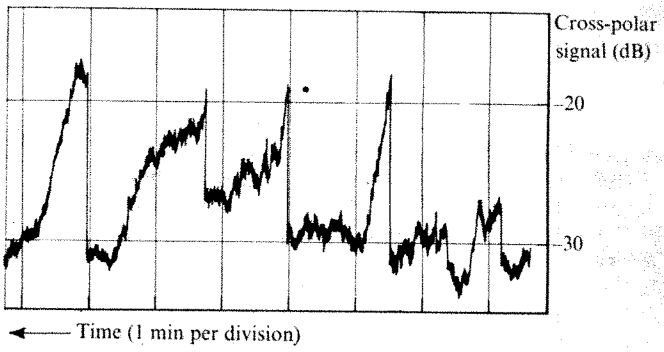

Fig. 1 Abrupt changes in cross-polarisation during a thunderstorm.

Following the observation of changes in cross-polarisation coincident with lightning strokes, a point-discharge electric field probe[7] and a photoelectric lightning detector were installed on site. The fine wire electrode of the field probe was 4 m above ground. Although no further lightning activity was recorded during satellite sessions, a strong correlation between anomalous cross-polarisation and point-discharge current was observed in three subsequent events, one of which, with a predominantly positive discharge current, is shown in Fig. 2. It is interesting to note that there is a better correlation between the probe current and cross-polarisation than between the co- and cross-polarised signals. In the other two events, a pronounced correlation between electric field on site and satellite path cross polarisation has been seen to occur with a time displacement.

To summarise, we have observed a pronounced correlation between electric field strength (measured near the ground) and cross-polarisation on a satellite radio path. Radar returns from high altitude (>3 km) particles, coincident with the appearance of high cross-polarisation, point

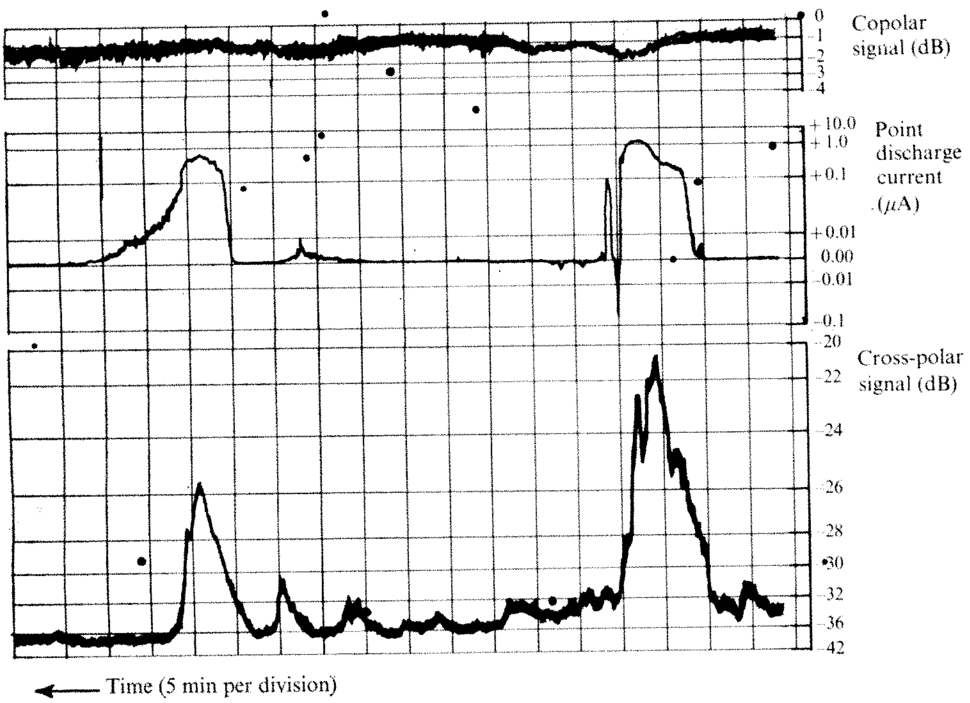

Fig. 2 Anomalous cross-polarisation and point-discharge current. Using an expression by Chalmers[7] the electric potential gradient, derived from the point-discharge current, is estimated at a maximum of 5 kV m^{-1} for the first peak and 1.7 kV m^{-1} for the second peak.

to ice particles as the cause for such cross-polarisation[1,2]. Analysis of the scattering from spheroidal ice particles[5,6] shows that high cross-polarisation can occur with small particles for low signal attenuation, provided that some degree of particle orientation is present.

Hendry and McCormick[8] have recently reported a high degree of orientation of ice particles from dual circularly polarised radar observations, which they have explained in terms of particle alignment in an electric field. 10^5 V m^{-1} has been shown to provide sufficient torque for rapid ($\sim$0.1 s) orientation of small ice spheroids[8].

We also interpret our results in terms of the orientation of small ice particles (1 mm or less) in the electric fields associated with convective storms. We emphasise that a degree of particle orientation does not necessarily imply a high degree of instantaneous alignment. Without significant damping forces imposition of an electric field on a randomly orientated group of ice needles or plates will produce simple harmonic motion around a preferred (average) orientation. The depolarisation from ice needles or plates[6] is such that only a small degree of preferred orientation is necessary for cross-polarisation.

In certain regions of storms aerodynamic turbulence will tend to disrupt field alignment, however it seems that in the upper regions of storm structures (typically $>$5 km in summer) turbulence is sufficiently low to permit a high degree of orientation[8]. Also the rapidity of alignment for small particles in strong fields, should permit realignment in the presence of turbulence provided that the latter is not overwhelming.

Alignment under aerodynamic forces alone is conceivable, but this does not explain the rapid lightning-correlated rises in cross-polarisation or the correlation between electric field and cross-polarisation.

The abrupt increases in cross-polarisation that we have observed may be interpreted in terms of the redistribution of charge in a thunderstorm at the incidence of a lightning stroke, resulting in a change in particle orientation. We emphasise that for linear polarisation a change in particle orientation with respect to the polarisation plane of the incident wave could result in either an increase or decrease in cross-polarisation, but a measurement with circular polarisation would show cross-polarisation related directly to the degree of particle orientation. Since coherent receivers were used for extraction of our cross-polarised signals (phase locked to the copolar signal), explanation of the abrupt cross-polarisation changes in terms of incoherent emissions from ionisation or lightning flashes is not credible.

If the rapid, lightning-correlated cross-polarisation changes that we have observed are caused by particle reorientation then we must conclude, from the observed orientation times, that small particles are responsible. Small particles are also inferred from the absence of significant attenuation during anomalous cross-polarisation[5,6].

The precise nature of electric fields in thunderstorms is a matter of current debate[9] and we hesitate to be dogmatic about the model applicable to our observations. Measurements of satellite cross-polarisation with circular polarisation would help clarify this issue. Apart from the implications of our results for the communications engineer, we would expect our results to be of relevance to the understanding of thunderstorm models.

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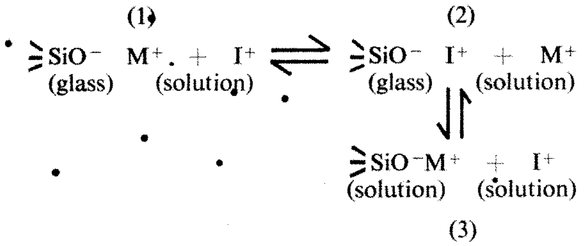
Decomposition of silicate glasses in alkaline solutions

WHEN a silicate glass comes into contact with a neutral or a slightly alkaline aqueous solution the only reaction which occurs is a diffusion controlled hydrogen–alkali ion exchange. The silica network is stable. In solutions with $pH >$ about 9, the network starts to break down at its interface with the solution. The rate of breakdown increases sharply with increasing pH; simultaneously the ion exchange rate decreases sharply. In solutions with $pH >$ about 12 it is difficult to detect the alkali released in solution by classical microanalytical methods as the solution is primarily made up of alkali hydroxide. It has been repeatedly suggested that the ion exchange reaction ceases to occur under these conditions and that the decomposition process becomes only a network breakdown. Surface and in-depth concentrations of alkalis in a glass which has been subjected to a highly alkaline solution have been determined by Auger electron spectroscopy (AES). Our results indicate that the ion exchange does not cease to occur in highly alkaline solutions as commonly believed.

The decomposition may be represented by the following scheme:



$$\begin{tikzcd}[column sep=small]
\underset{\text{(glass)}}{\geqslant\text{SiO}^-\ \text{M}^+} + \underset{\text{(solution)}}{\text{I}^+}\ \text{(1)} \arrow[r, shift left] & \arrow[l, shift left] \underset{\text{(glass)}}{\geqslant\text{SiO}^-\ \text{I}^+} + \underset{\text{(solution)}}{\text{M}^+}\ \text{(2)} \arrow[d, shift left] \\
 & \arrow[u, shift left] \underset{\text{(solution)}}{\geqslant\text{SiO}^-\text{M}^+} + \underset{\text{(solution)}}{\text{I}^+}\ \text{(3)}
\end{tikzcd}$$

where M denotes an alkali atom, usually sodium, while I denotes a solvated monovalent cation, usually a proton. This reaction scheme is widely used in discussing the physicochemical processes (corrosion, membrane potentials, electrokinetic phenomena, etc.) exhibited by glass and by many silicates.

The scheme often appears as two separate equations relating (1) ⇌ (2) and (2) ⇌ (3). Ion exchange processes which take place in aqueous solutions and involve a solvated proton as a reacting species are essentially acid–base reactions. Reactions (1) ⇌ (2) and (2) ⇌ (3) are related through the equilibrium constants of the conjugate

$$\geqslant\text{SiOH}/\geqslant\text{SiO}^-$$

acid–base pair[1] by the relation $K_aK_b = K_w = 10^{-14}$ at 25 °C, K_a being of the order 10^{-10}, $K_b \sim 10^{-4}$.

In alkaline solutions where $a_{M^+} \geqslant a_{OH^-} \gg a_{H^+}$ a shift of the equilibrium towards positions (2) and (3) is favoured. The reverse occurs in acidic solutions. Studies on the kinetics of approach to the equilibrium of reaction (1) → (2) and (2) → (3) have been carried out in aqueous solutions buffered at pH values of 1–13. For pH of 1–9 the rate of dealkalisation remained constant, and independent of the pH of the solution, and sharply decreased with increasing pH thereafter. The rate of breakdown was extremely low in the pH range 1–9 and sharply increased with pH above the critical value of about 9 (ref. 1).

Chemical analysis of alkalis and silica in the glass and in solution, at various pH values, shows that the ratio M_2O/S_iO_2 is always higher in acidic, neutral and slightly alkaline solutions than in the glass. This is considered[1] to imply that reaction (2) → (3) lags behind reaction (1) → (2) and a silica-rich leached layer is developed on the glass surface in contact with the solution. The existence of such a layer has been proved[2] by the establishment of in-depth composition profiles of glasses which have been in contact with aqueous solutions of varying pH.

As the pH of the solution increases above 9, the M_2O/SiO_2 ratio in the solution slightly decreases and tends to approach the same value as in the glass. Hence, it has been inferred[3–6] that the decomposition of glass becomes entirely a dissolution process, that is, a network breakdown as represented by reaction (2) → (3). This inference is tempting, because it appears difficult for a solvated alkali ion in solution to re-enter the glass by reaction (2) → (1) to occupy a site originally filled by an anhydrous alkali ion. The almost linear rate of decomposition of glass in highly alkaline solutions has been considered to support such inference, but concrete evidence is lacking. Analytical solution chemistry is little value for deciding whether reaction (2) → (3) goes faster than reaction (2) → (1) or continues to lag behind it because one would have to detect parts per million of the alkali species originally present in the glass in the presence of much higher concentration of the other alkali species which is used for buffering the medium. We have devised the following experiment to decide whether the breakdown of glass represented by reaction (2) → (3) supersedes the ion exchange reaction (2) → (1) under the extreme condition where $a_{M^+} > a_{OH^-} \gg a_{H^+}$. Two identical fresh rectangular samples were prepared by breaking down a commercial soda-lime–silica glass container which has not been subjected to any surface treatment. The approximate composition in weight per cent of the glass was as follows: 74% SiO_2, 13% Na_2O, 12% ($MgO+CaO$) and 1% other oxides. The first sample was immersed in a solution of 0.1 M NaOH; the second in a solution of 0.1 M NaOH+1.0 M Li_2CO_3. Each sample was allowed to remain in contact with the solution for 5 h at 25 °C. Lithium was chosen as a tagging cation as the free energy change of its association with the $\rightarrow SiO^-$ sites makes it more favourable to other alkalis[5]. If reaction (2) → (3) proceeds faster than reaction (2) → (1), lithium could not be detected on the treated glass surface, and complete dissolution of the glass in highly alkaline solutions, already inferred by many workers, would become evident. If, on the other hand, Li^+, not in association with carbon, from the CO_3^{2-} radical, with which it must be at least partly associated in solution, could be detected in the glass to

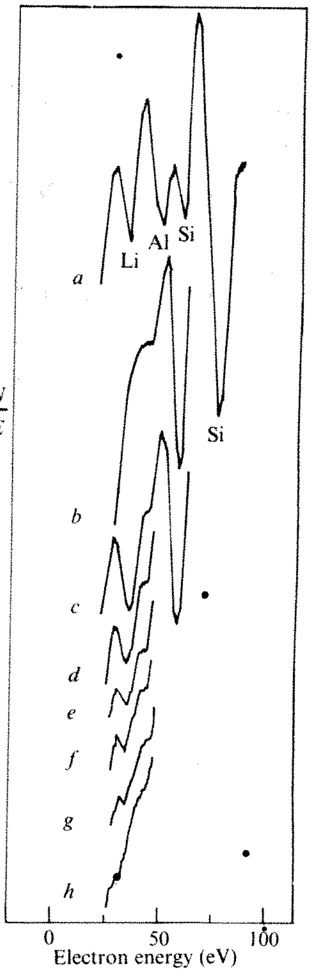


Fig. 1 Auger electron spectra for (*a*) Spodumene fracture surface (*b*) Soda-lime–silica glass surface treated in 0.1 NaOH (*c*) Soda-lime–silica glass treated in 0.1 N NaOH+1.0 N Li_2CO_3. (*d–h*) Sample *c* after bombardment with a 2.0 keV beam of argon ions for 0.5, 2.0, 6.0, 10.0 and 18.0 min, respectively. Beam voltage, 3 kV; beam current, 3μA (~ 250 μm diameter); modulation amplitude, 5 eV; scan rate (0–100 eV), 2 eV s^{-1}; time constant, 1 s; sensitivity, 1 μV.

some depth, this would indicate a decomposition process in which the ion exchange reaction still has a role.

$$\underset{\text{(glass)}}{\geq\text{SiO}^-\text{Na}^+} + \underset{\text{(solution)}}{\text{Li}^+} \rightleftharpoons \underset{\text{(glass)}}{\geq\text{SiO}^-\text{Li}^+} + \underset{\text{(solution)}}{\text{Na}^+}$$

AES was performed on the two glass samples treated in the manner outlined above. The technique used was that described by Pantano *et al.*[2] for glass surfaces. It involves cooling the samples to liquid nitrogen temperature, maintaining the beam current density at low value and minimising the electron irradiation time. Approximate surface and in-depth concentration of alkalis were determined from the spectra using an Ar ion-milling apparatus to remove surface material while simultaneously analysing with AES. The change in the Auger peak-to-peak height of each species is generally assumed to be proportional to the change in concentration of that constituent. Figure 1 shows the spectra obtained.

The spectra of a spodumene ($Li_2O.Al_2O_3.4SiO_2$) fracture surface was obtained as a standard, because of the difficulty of identifying the Auger transition for Li (Fig. 1*a*). The peak energy for the Auger transition assigned to Li is 37 eV, while the main Si peak appears at the 76 eV with a minor peak at 58 eV, and the Al peak appears at 51 eV. In Fig. 1*b* the only Auger transition in this energy range is due to Si (58 eV). The main silicon peak at 78 eV is not shown. In Fig. 1*c* in addition to Si, the peak identified as being due to an Li Auger transition is also detected. In Fig. 1*d*–*h*, the persistence of a Li signal for up to 10 min of ion milling (~ 250 Å) suggests the existence of Li within the glass surface. Also, the sodium peak intensity of the surface treated with $NaOH+Li_2CO_3$ was approximately 40% less than that measured on the glass treated without Li. This is a reasonable result as the sites now occupied by Li would otherwise be occupied by Na. These results lead us to exclude complete dissolution, or glass network breakdown, as a possible mechanism for glass decomposition in these solutions and conclude that the ion exchange continues to have an important role even in highly alkaline solutions.

Ceramic materials and more than two thirds of the rocks constituting the Earth's crust are basically made of the same building unit as glass, that is, the silicon–oxygen tetrahedron, and consequently undergo the same sequence of reactions with aqueous solutions. The present conclusion possibly applies to other silica-based materials.

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A near perfect shape-memory ceramic material

After high temperature plastic deformation and cooling under load to room temperature, glass-ceramics containing mica as the principal crystalline phase retain a near-perfect memory of their undeformed state, the plastic strain being recoverable by a subsequent high temperature anneal. Activation energy measurements support the hypothesis that dislocations in the mica crystals control the shape-memory process.

The elastic martensitic transformations responsible for shape-memory phenomena in certain metal alloys have been the subject of extensive research, recently reviewed by Wayman[1]. In principle, any multi-phase system containing phases with different viscosities (but equal shear moduli) can also exhibit shape-memory behaviour since elastically strained components are capable of driving viscous elements back towards their original undeformed shapes. Such behaviour, by internally oxidised copper, has been demonstrated by Brown and Stobbs[2].

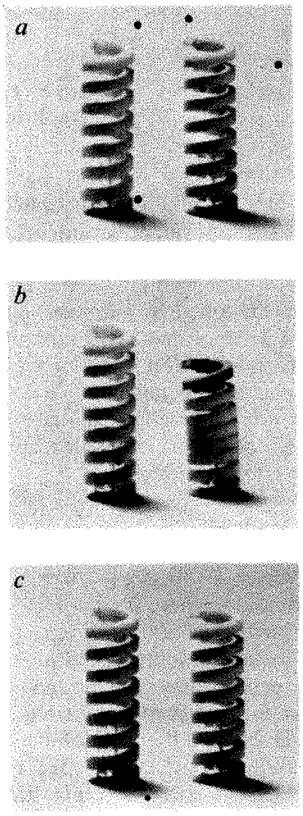

Fig. 1 The shape-memory behaviour of a mica glass-ceramic. The specimen on the right has been subjected to high temperature compression before unloading at room temperature (*b*) and then annealed at high temperature (*c*).

At temperatures >300 °C, mica can be deformed plastically by slip between its basal layers. Such deformation in crystals intimately mixed with a rigid, multiply connected phase should permit retention at room temperature of any small shape change imposed on the phase mixture at temperatures above 300 °C; since glide in mica is not possible at low temperature, removal of the mechanical constraint, after cooling to room temperature, is not accompanied by the reversal of plastic strains in the mica that would be required in order to release fully the elastic strain in the matrix. The elastic strain energy thus stored provides a driving force for recovering the undeformed shape, should the temperature subsequently approach 300 °C. If the micaceous phase is singly connected, no plastic strain can leak to the free surface and, in principle, the phase mixture should be capable of returning precisely to its original shape.

The series of mica glass-ceramics developed at Corning Glass Works[3] fit this specification for a perfect shape-memory solid. The volume fraction of fluorophlogopite, the

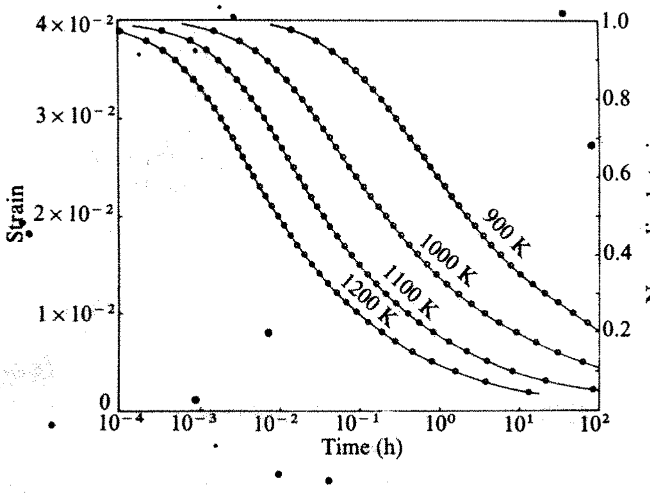

Fig. 2 Summary of torsional strain recovery data obtained from Corning 9650.

main crystalline phase, is typically 2/5 to 3/5, with maximum crystal diameters ranging from ¼ µm to 100 µm and aspect ratios of order 10 : 1. The mica glass-ceramics are easily machined using conventional tools, and helical specimens having outer diameter 2 cm and pitch 3.2 mm were cut from as-crystallised rods. Figure 1 shows two identical helical specimens machined from Corning code 9650, (*a*) before deformation, (*b*) after quickly cooling the specimen on the right from axial compression at 500 °C and (*c*) after subsequently annealing (*b*) for 2 h at 800 °C. Each individual pitch has recovered at least 87% of the imposed strain. In other preliminary experiments, recoveries of 99% after prolonged anneals have been attained.

Using improved specimens ultrasonically machined from Corning codes 9650 and 9658, accurate measurements of axial strain recovery have been made during annealing at several temperatures. The measurements have been converted to local torsional strain and Fig. 2 shows a summary of the data for Corning code 9650. Similar data was obtained for glass-ceramic code 9658. For each material, the data from all temperatures may be superposed on to a master curve at, say, 1,000 °C merely by shifting the individual curves horizontally. Assuming that a single mechanism is rate controlling, the shift corresponds to an activation energy of 42±5 kcal mol⁻¹ for Corning 9650, and 22±3 kcal mol⁻¹ for Corning 9658. These are much lower than the activation energies for large scale, high temperature creep of the same mica glass-ceramics. In the temperature range 480–670 °C, for example, Corning 9650 tested in tension creeps with an activation of 100 kcal mol⁻¹ (James and Ashbee[4]). The larger activation energy is characteristic of flow of the glassy matrix, whereas the activation energy for shape-memory behaviour is expected to be that for dislocation glide in mica. A measurement for the latter, obtained from the temperature/time dependence of the microhardness of muscovite shown in Fig. 3, is 29±4 kcal mol⁻¹.

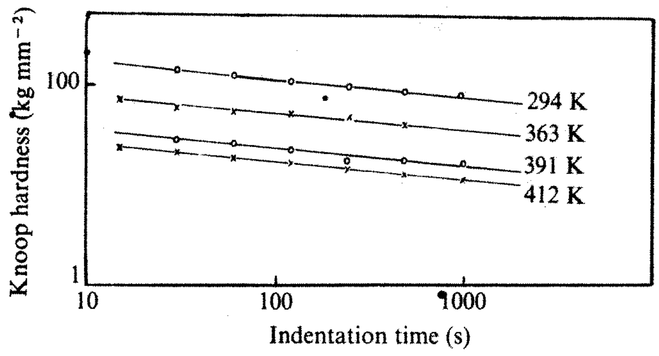

Fig. 3 Indentation creep of muscovite single crystals. (Long axis of Knoop indenter parallel to *a* axis.)

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The use and misuse of pure water PVT properties for lake waters

ALTHOUGH dissolved substances affect the thermodynamic properties of lake waters,[1,2] many limnologists still consider lake water as pure water[3–8]. This can be a good assumption if used with caution: lake water is by no means pure water, especially when the precise pressure, volume, temperature (PVT) properties are considered. PVT properties of lake water can be determined from an equation of state for seawater provided that the total mass fraction of dissolved salts in seawater and lake water are equated[9]. In addition, the total mass fraction of dissolved salts affects calculations concerning the temperature of maximum density and thus stability[10]. Therefore, in the absence of direct PVT measurements on a particular lake, it is at present most appropriate to use an equation of state for seawater when examining effects of PVT properties in lakes. We present here a short equation of state determined with a precision of 2×10^{-6} g cm⁻³ in density over the appropriate range for most fresh water lakes, 0–0.6‰ salinity, 0–30 °C, and 0–180 bar. The equation of state is based on the 1 atm pure water density and compressibility equations of Kell[11], the pure water temperature and pressure effects of Fine and Millero[12], and the effects of dissolved salts of Millero *et al.*[13] and Chen and Millero[14]:

$$d^{\mathrm{P}} = d^0/(1-P/K) \tag{1}$$

$$\begin{aligned} d^0\,(\mathrm{g\,cm^{-3}}) = {} & 0.9998395+6.7914\times10^{-5}t \\ & -9.0894\times10^{-6}t^2+1.0171\times10^{-7}t^3 \\ & -1.2846\times10^{-9}t^4+1.1592\times10^{-11}t^5 \\ & -5.0125\times10^{-14}t^6+(8.221\times10^{-4} \\ & -3.87\times10^{-6}t+4.99\times10^{-8}t^2)\,S(‰) \end{aligned} \tag{2}$$

$$\begin{aligned} K(\mathrm{bar}) = {} & 19652.17+148.376t-2.329t^2 \\ & -1.3963\times10^{-2}t^3-5.90\times10^{-5}t^4 \\ & +(3.2918-1.719\times10^{-3}t+1.684\times10^{-4}t^2)P \\ & +(-0.8985+2.428\times10^{-2}t+1.114\times10^{-2}P)\,S(‰) \end{aligned} \tag{3}$$

where d^{P} and d^0 are the densities of lake water at applied pressures

P and 0 (sea level), respectively, t is the temperature in °C, $S(‰)$ is the salinity:

$$S(‰) = 0.9951437 g_T \quad (4)$$

where g_T is the total grams of dissolved salt in 1 kg of lake water[15]. The values of g_T usually increase with depth due to the increase of HCO_3^-, Ca^{2+} and nutrients[16]. The small changes in composition in a lake can be accounted for by making the appropriate changes in g_T. Further, Millero[9] and Millero *et al.*[17] showed that the densities calculated from the seawater equation of state for Lake Tanganyika water and artificial river water agree with the measured values to within $\pm 2 \times 10^{-6}$g cm^{-3} despite the large composition difference between the two waters. This verifies the validity of our equation of state for fresh waters.

One atmosphere densities computed from equation (1) at 0.15, 0.30, and 0.60‰ salinity over the temperature range of 0–30 °C are shown in Fig. 1. Also shown are the densities for pure water at 0 and 1.0 bar (gauge). It is clearly shown that even a nominal amount of salt, 0.15‰ salinity or 151 mg kg^{-1}, increases the density of pure water appreciably. At 0 °C, the increase in density is 123×10^{-6}g cm^{-3}, which is equivalent to a change of 2.4 bars in pressure or 2.9 °C in temperature. This increase in density has often been neglected and it is certainly not negligible.

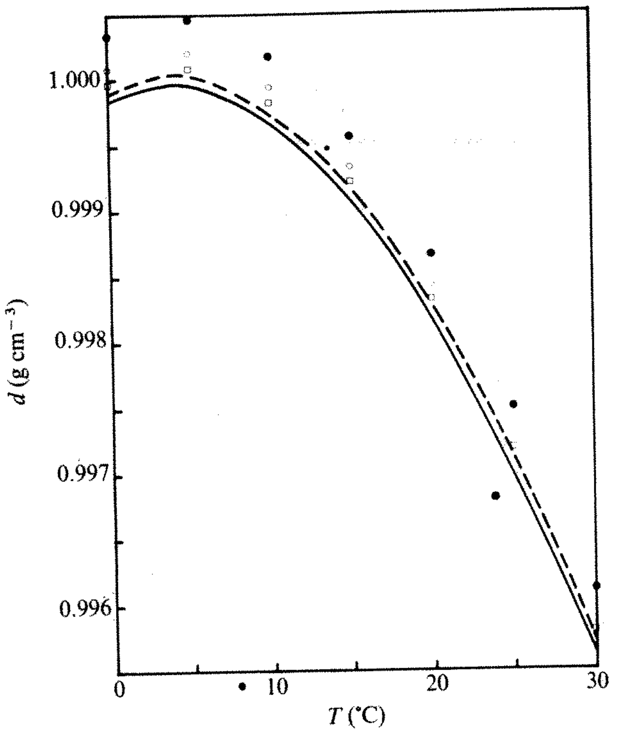

Fig. 1 Densities for pure water at 0 (———) and 1.0 (– – –) bar (gauge) and for lake waters at 0.15 ( ), 0.30 ( ) and 0.06 ( ) ‰ salinity at 0 bar (gauge) over 0–30 °C.

The existence of a temperature of maximum density, T_{MD}, is of fundamental importance in limnology. An equation is given to be used to estimate T_{MD} with a precision of 0.001 °C,

$$T_{MD} = 3.9839 - 1.9911 \times 10^{-2}P - 5.822 \times 10^{-6}P^2 - (0.2230 + 1.111 \times 10^{-4}P)S(‰) \quad (5)$$

At 1 atm, a nominal amount of salt, 150 mg kg^{-1}, in a freshwater lake contributes 0.033 °C to the depression of the temperature of maximum density. This seemingly small number, 0.033 °C, which corresponds to the effect of 16.8 m of water, is orders of magnitude larger than the precision of the *in situ* temperature measurements and certainly should not be neglected. We, therefore conclude that the salt content is of significance in freshwater lakes and our equation of state should be used to calculate reliable PVT properties for lake waters.

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Raman scattering and the characterisation of atmospheric aerosol particles

AIRBORNE particulates have a major role in the air pollution problem. They are responsible for acid rain, reduced visibility, and, in certain size ranges, are deposited in the lungs where they can cause various disorders. Considerable effort has been expended in the analysis of these particulates using techniques such as wet chemistry, X-ray fluorescence, infrared spectroscopy and ESCA. Yet there are still great uncertainties as to the chemical form and origin of many of the particulate species. We describe here preliminary studies of the feasibility of characterising particulate pollutants by means of Raman spectroscopy. We believe that this is the first attempt to apply this spectroscopic technique in this area of research. The samples studied included diesel exhaust particles, automobile exhaust particles (unleaded fuel, no catalytic converter) and several ambient air samples. The spectra from these samples were compared with those of polycrystalline graphite and activated carbon which were used as references. Our results indicate that physical structures similar to activated carbon are at least a major species and may be the dominant species in both source-enriched and ambient samples. The implications of this observation could be quite important in terms of atmospheric chemistry, health effects, weather modifications and the effects of aerosols on the albedo.

The beam from a Coherent Radiation argon ion laser (operating with 1 W at 5145 Å) was focused by a 75-mm focal length cylindrical lens to a spot of 0.06 × 2 mm on the sample surface, and the backscattered radiation was collected and imaged by an f/3.4 lens on to the slit of a Jarrell Ashe double monochromator. The incident polarisation of the laser was perpendicular to the slit of the spectrometer, and the scattered polarisation was not analysed. The output of the spectrometer was detected by an FW130 photomultiplier cooled to −20 °C and used in a photon-counting mode. The photon pulses, after appropriate pulse shaping, were counted and displayed on a multichannel analyser. To minimise heating effects, the highly absorbing samples used in these experiments were rotated at 1800 r.p.m. by a motor—this increases the area illuminated by the laser beam by a large factor with almost no loss in signal level. The focal spot of the laser was located approximately 5 mm below the axis of rotation so that the effective illuminated area was an annulus of radius 5 mm and width 2 mm which yielded a rather low power density of ~ 1.5 W cm^{-2}.

The samples used in these experiments were EDM 3 grade polycrystalline graphite (POCO Graphite Inc.), Norit A activated carbon (Pfanstiehl Labs), diesel exhaust collected on a glass fibre filter, automobile exhaust collected on a glass fibre filter from a number of cold starts of a poorly tuned 1974 Ford Pinto (lead-free fuel; no catalytic converter), and an ambient samples collected in 1975 as part of EPA's RAPS programme in St. Louis, Missouri. The latter sample was collected on a 1.2-μm Millipore filter using a dichotomous sampler[1] and was in the small size range fraction. The diesel and car exhaust samples were removed from the filter and transferred to an aluminium flat. This technique allowed us to obtain excellent Raman spectra of the particulates without interference from the large fluorescence of the filter material. This technique could not be applied to the ambient samples because of insufficient loading, and therefore in this case the Raman spectrum was obtained directly from the particles on the Millipore filter substrate.

Figure 1 shows the Raman spectrum obtained from automobile exhaust. Similar spectra were observed for diesel exhaust, activated carbon, and polycrystalline graphite with the dominant features of the spectrum occurring at 1,200–1,700 cm^{-1}. For polycrystalline graphite a strong line is also observed at ~2,700 cm^{-1} which we associate with a C–H vibration. In the ambient sample, where the signal to noise is severely limited by the large fluorescence background, the only lines that were clearly seen above the noise level were also in the 1,200–1,700 cm^{-1} spectral region. Figure 2 shows the Raman spectrum for the various samples in this region.

Koenig *et al.*[2] measured the Raman spectrum of a single crystal of graphite as well as that of polycrystalline graphite and activated carbon. In a single crystal only the Raman mode near 1,600 cm^{-1} is observed, and from a group theoretical analysis, it has been assigned to the $k = 0$ E_{2g} phonons of the graphite lattice. The mode near 1,350 cm^{-1} appears only in samples which are not perfectly ordered, and its intensity relative to the one near 1,600 cm^{-1} varies inversely with the crystallite size, L_a, as obtained from X-ray data. Solin *et al.*[3] have shown that this ratio tends to saturate for crystallite sizes less than 40 Å. The identification of the 1,350 cm^{-1} mode is uncertain, but Koenig *et al.* suggest that it may be due to an A_{1g} phonon which is normally Raman inactive but is active in this case due to a breakdown of the k selection rules by the small crystallites in the sample.

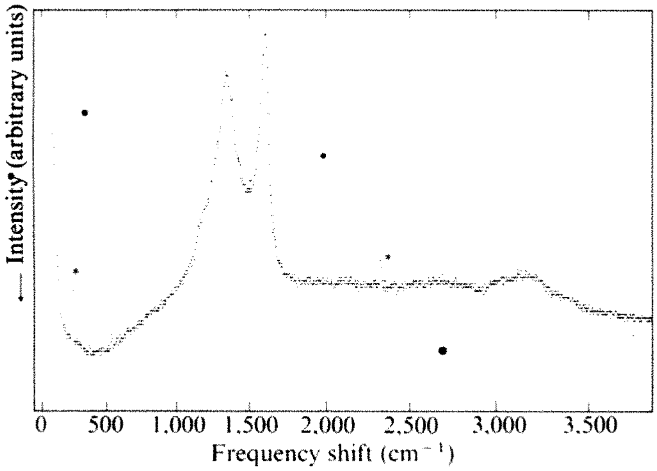

Fig. 1 Raman spectrum of automobile exhaust in the spectral region between 90 and 3,830 cm^{-1}. The slit width for this scan was 3 Å. The lines identified with an asterisk are due to grating ghosts.

It is evident from Fig. 2 that the spectra of activated carbon, diesel exhaust, automobile exhaust, and the ambient sample are very similar. The positions of the two Raman modes in these spectra are coincident to within ±10 cm^{-1} which is the estimated experimental error. Since the phonon frequencies are a sensitive probe of the lattice, we suggest that these spectra give strong evidence for the existence of physical structures similar to activated carbon in the samples we have studied. This is not surprising since activated carbon is also produced in a combustion process.

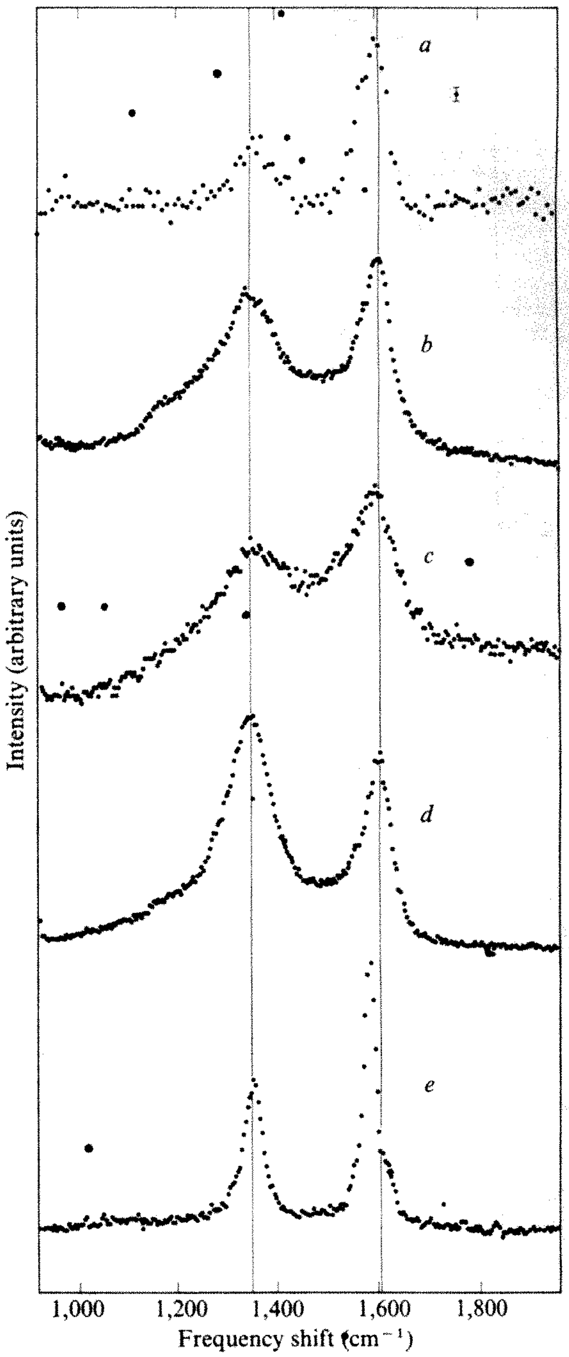

Fig. 2 Raman spectra between 1,200 and 1,700 cm^{-1} of *a*, ambient sample; *b*, automobile exhaust; *c*, diesel exhaust; *d*, activated carbon and *e*, polycrystalline graphite. The slit width for samples *b*–*e* was 3 Å; for sample *a*, 7-Å slits were used to improve signal to noise ratio.

We can estimate from literature data[2] the crystallite sizes in the various samples from the intensity ratios of the two observed Raman modes. The automobile exhaust and diesel exhaust samples have roughly the same peak intensity ratio, yielding crystallites of ≃ 50 Å in size, while the ambient sample seems to have crystallite sizes of ≃ 100 Å. We would expect such structures to have very large internal surface area.

Novakov *et al.*[4] have proposed that fine soot particles, which in many ways are similar to activated carbon, may have a major role in atmospheric chemistry. To assess the importance of the proposed soot-catalysed reactions, it is important to determine the chemically active primary carbon in the carbonaceous fraction of ambient and source-enriched aerosol particles. Our results indicate that physical structures similar to activated carbon are present in diesel exhaust, automobile exhaust and ambient samples. The fact that these features tend to dominate the Raman spectrum may indicate that graphitic 'soot' is the major species in the samples we have measured. But, the large intensity in these modes may be due just to their large Raman cross-section, and therefore a quantitative interpretation of these results will have to await a more detailed analysis. Such an analysis will have to include measurements of the Raman cross-section, optical absorption cross-section, and take into account the little-understood particle size effects[5].

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Fluctuations of seismicity before major earthquakes

LONG-TERM precursory changes of seismicity previously reported are (1) a change in the relationship between earthquake frequency and magnitude, and (2) a decrease in the frequency of micro-earthquakes. In a review of earthquake precursors Rikitake[1] cites eleven examples of the former and three examples of the latter. On the dilatancy hypothesis a decrease in activity is to be expected from dilatancy hardening[2]; an earlier gradual increase of activity due to the secular increase of tectonic stress was also postulated[2]. A more distinctive sequence of seismicity fluctuations can be recognised from data in seismological bulletins. The fluctuations are large: a burst of activity marking the start of the precursory sequence is followed by a period of abnormal quiet, and this lasts until the onset of the major event. We report here characteristic changes of minor earthquake activity, preceding a number of recent major earthquakes in regions with good seismographic coverage, and suggesting a new basis for long-term prediction relying directly on the normal data output of permanent seismograph networks.

The initiating burst of activity differs markedly from the major event: the general level of magnitude is substantially lower, and no earthquake is much larger than the rest. Thus the term 'precursory swarm' is appropriate, for although earthquake swarms are commonly regarded as characterising regions of Quaternary volcanism it is the lack of a main shock that distinguishes a swarm from a typical tectonic event. The abnormally low activity with which the precursory sequence continues defines a seismic gap in both time and space; the geographic gap is of premonitory value since the major event (as well as the precursory swarm) occurs within it, though whether it is well delineated depends on the ambient level of seismicity and the accuracy of epicentre location.

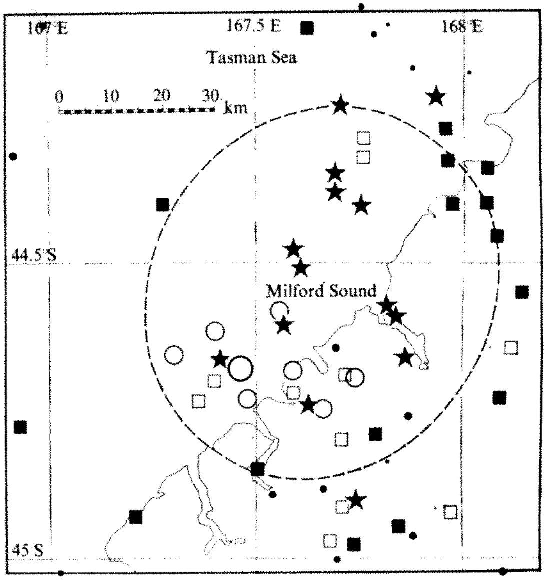

Fig. 1 Epicentre map of crustal earthquakes with $M_L \geqslant 4.6$, occurring from January 1961 to May 1976, and showing approximate boundary of precursory area for the Milford Sound earthquake of 4 May 1976, $M_L = 7.0$. The seismic episodes indicated are as follows: normal seismicity (□), 1 Jan. 1961–21 Feb. 1968; precursory swarm (★), 22 Feb. 1968–23 Dec. 1968; precursory gap (■), 24 Dec. 1968–3 May 1976; major event (○), 4 May 1976–8 May 1976.

The phenomenon is illustrated by two major earthquakes, one from New Zealand and the other from California. The Milford Sound earthquake of 4 May 1976, magnitude $M_L = 7.0$, is the largest New Zealand shock since 1968, although by virtue of its location offshore in Fiordland, a rugged and sparsely populated region, it attracted little public notice. The Ocotillo Wells earthquake of 9 April 1968, magnitude $M_L = 6.4$, was one of the two largest California earthquakes since 1954; it was widely felt and involved displacements along a 33-km segment of the Coyote Creek fault[3].

The Milford Sound earthquake data are taken from published bulletins[4], and from recent files made available by Dr R. D. Adams, Superintendent, Seismological Observatory, D.S.I.R., Wellington; the data for the Ocotillo Wells earthquake are taken from Hileman *et al.*[5].

For the relevant areas, Figs 1 and 2 show the epicentres of located earthquakes occurring in the specified time intervals

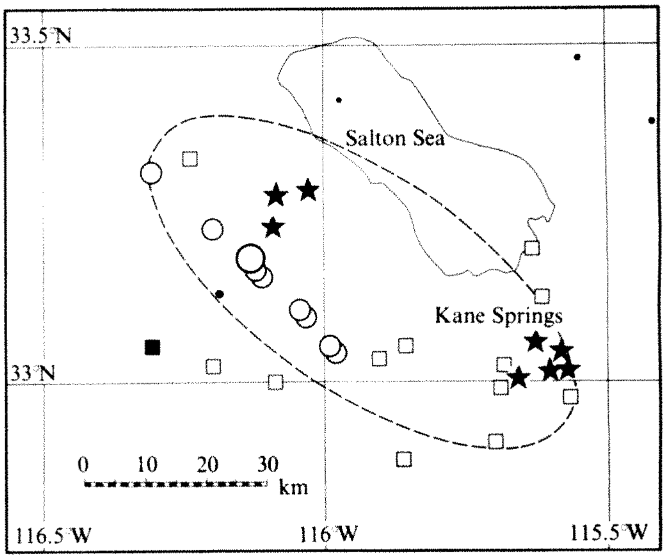

Fig. 2 Epicentre map showing approximate boundary of precursory area for the Ocotillo Wells earthquake of 9 April 1968, $M_L = 6.4$. All earthquakes with $M_L \geqslant 4.1$, occurring from January 1962 to April 1968 are shown. The seismic episodes indicated are as follows: normal seismicity (□), 1 July 1962–10 April 1965; precursory swarm (★), 11 April 1965–26 August 1965; precursory gap (■), 27 August 1965–8 April 1968; major event (○), 9 April 1968–16 April 1968.

and magnitude ranges. (The Milford Sound major event was crustal in origin and earthquakes originating below the crust have been excluded.) Four seismic episodes are distinguished: normal seismicity, the precursory swarm, the precursory gap and the major event.

The hypothesis that all the swarm and major-event epicentres and none of the gap epicentres lie within a certain area is satisfied by the elliptical boundaries shown in Figs 1 and 2; in the Milford Sound example allowance is made for location errors of up to 7 km, which is within the typical standard deviation for epicentre locations in the region. Of course, any actual boundary will not be precisely elliptical. It is of interest, however, that in each example the orientation of the ellipse is approximately parallel to the dominant tectonic trend in the region.

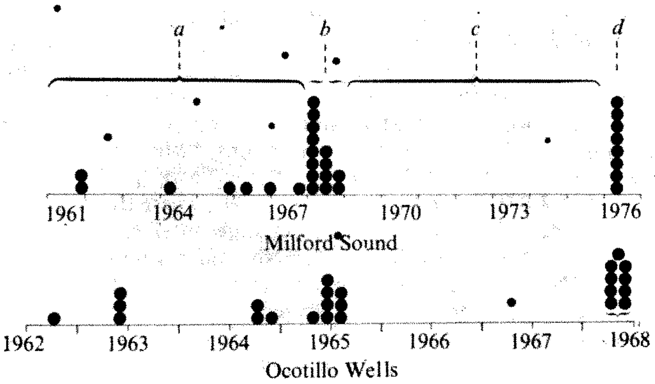

Fig. 3 Seismic sequences associated with the precursory areas of the Milford Sound and Ocotillo Wells earthquakes. The period of normal seismicity has been chosen about equal to that of the precursory gap, and the two time scales have been adjusted to facilitate comparison. *a*, Normal seismicity; *b*, precursory swarm; *c*, precursory gap; *d*, major event.

Thus the fluctuations of seismicity follow a simple geographic pattern. For the Ocotillo Wells sequence the precursory boundary encloses most of the normal seismicity and it is thus not surprising that the precursory gap episode is not represented by more epicentres.

The seismic sequences are plotted against time in Fig. 3, which shows how the seismicity within the precursory area fluctuates, in relation to the normal level, before a major event.

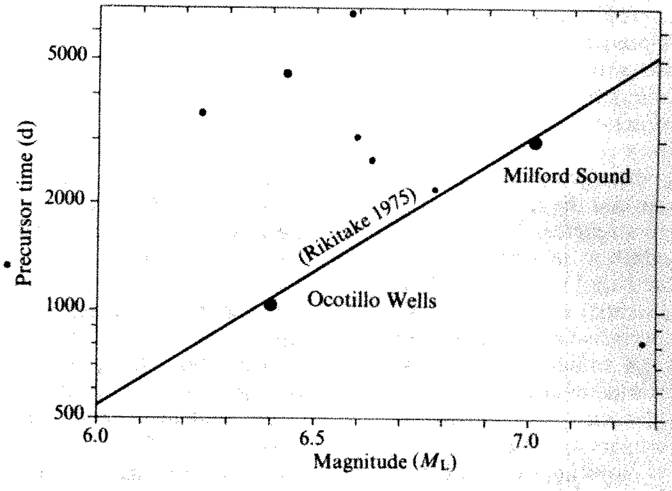

Fig. 5 Time interval between precursory swarm and major event, plotted against main-shock magnitude. Rikitake's curve is obtained from a plot of widely scattered data for eleven different types of precursory phenomena: land deformation, *b*-value, micro-earthquakes, source mechanism, fault creep, velocity ratio, velocity values, geomagnetic effects, resistivity, radon emission and oil flow.

The precursory swarm could be described as a rehearsal of the major event, but the gap is also an important feature of the precursor.

The premonitory value of the fluctuations of seismicity depends in the first instance on the successful recognition of a swarm as such. Magnitude level is no criterion as major events in the present sense of the term also occur at low magnitudes. The required criterion is provided by the distribution of magnitudes and, in particular, as mentioned above, by the absence of a main shock. This is illustrated in Fig. 4, which contrasts the two precursory swarms with the respective major events.

Taking the precursor time as the interval between swarm and major event (onset-to-onset) one obtains 2,994 d for the Milford Sound earthquake and 1,029 d for the Ocotillo Wells earthquake. These values are plotted against the magnitudes of the respective main shocks in Fig. 5. Also shown is the relationship obtained by Rikitake[1] based on other types of precursor.

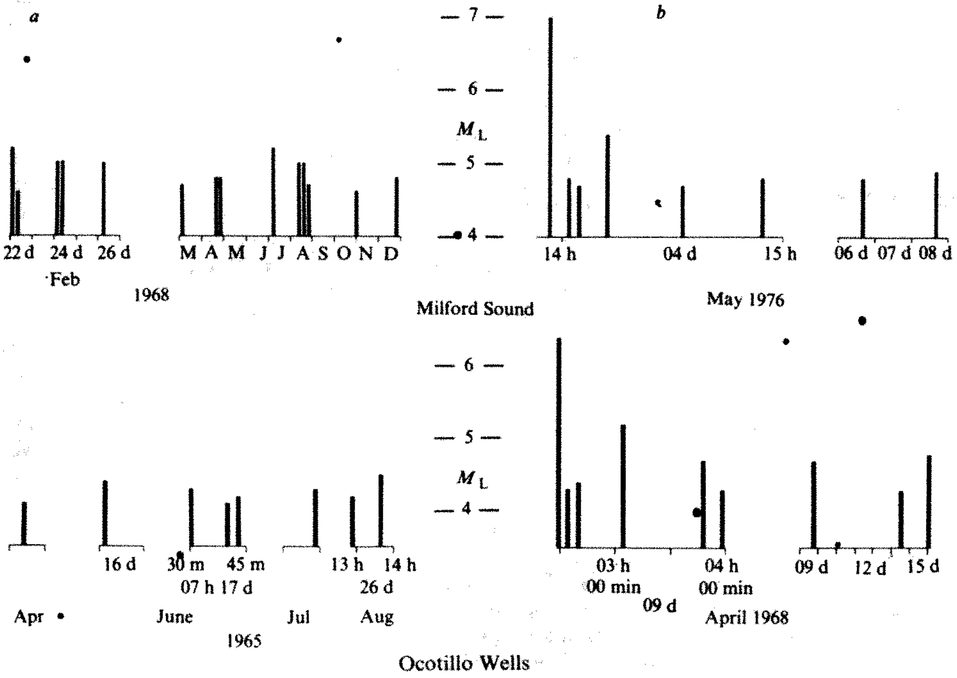

Fig. 4 Magnitudes of precursory swarm (*a*) and major event earthquakes (*b*). The time scales are not uniform.

The fluctuations of seismicity seem to have a physical origin closely related to that of the phenomena analysed by Rikitake. As with many of those phenomena the dilatancy hypothesis offers an interpretation of the precursory swarm. For at the onset of dilatancy the rock undergoes pervasive cracking which in an inhomogeneous medium can be expected to involve a redistribution of stress, leading in some localities to an increase beyond the strength. But as the general effect of the cracking is to strengthen the rock, only comparatively minor earthquakes result at this stage. The mechanism of the well-known swarm activity in regions of Quaternary volcanism may be similar in essential respects, as this activity may also be associated with pervasive cracking as the rock cools and shrinks[6].

A strategy of earthquake prediction has been suggested by Rikitake[1] in which an extremely long-range precursor would be identified by repeated geodetic surveys, and long-range precursors would then be sought by intensive geophysical methods. It now appears that fluctuations of seismicity may provide long-range precursory information from the normal output of a permanent seismograph network, without the use of intensive methods. Such methods could then be devoted to the search for short-term precursors.

Seismicity fluctuations have been identified in association with main shocks at magnitudes from 5.0 to 7.1. The phenomenon provides the following precursory information: the approximate location of the future major event is indicated at a prior time which is related to the magnitude of the main shock, and the area affected, although not always well defined, is also related to the magnitude. In addition, however, it seems that with the seismicity fluctuations the magnitude of the main shock increases with that of the swarm earthquakes; this relationship is potentially of great premonitory value and is now being investigated. Present studies are also leading to a detailed formulation of the characteristics of precursory swarms and to an assessment of the seismographic coverage required by the method at given levels of main-shock magnitude. On the basis of these studies the reliability of the method is being investigated preparatory to attempting actual predictions.

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Muon production of ^{92,94}Nb in the Earth's crust

ACTIVITIES due to long-lived ^{92}Nb ($t_{1/2} \simeq 1.7 \times 10^8$ yr) and to ^{94}Nb ($t_{1/2} = 2.03 \times 10^4$ yr) in a refined bar of terrestrial niobium were discovered by Apt *et al.*[1]. They speculated that the ^{92}Nb activity could have applications to chronologies of nucleosynthesis and to cosmogony. As an example, they calculated that their observed activity level would result if the isotopic ratio 4.7×10^9 yr ago were $^{92}\mathrm{Nb}/^{93}\mathrm{Nb} = 10^{-3}$ and if the halflife were 1.58×10^8 yr. More definite conclusions were difficult because the concentration expected today depends so strongly on the poorly known halflife, which they estimated to be 1.7×10^8 yr from a reactor irradiation. This sensitivity can be illustrated by noting that a 5% error in halflife corresponds after 4.7×10^9 yr to an error of a factor 2.74 in the anticipated activity. Quantitative calculations from galactic nucleosynthesis must therefore await a more precise measurement of the halflife. Implications of primordial ^{92}Nb have recently been enlarged with the proposal[2] to use enrichments $\delta(^{92}\mathrm{Zr})$ in the isotopic composition of Zr due to extinct ^{92}Nb to measure absolute time differences in the formation of meteorites and planets. If an initial $^{92}\mathrm{Zr}/^{94}\mathrm{Zr}$ ratio for each body can be obtained, differences between those initial values could be translated into time differences in the formation of the respective objects. We argue here that both projects are probably doomed to disappointment. It seems to us unlikely that the near equality in the two measured activities is a coincidence. They are respectively 0.058 ± 0.035 d.p.m. per kg (Nb) for ^{92}Nb and 0.32 ± 0.03 d.p.m. per kg (Nb) for ^{94}Nb. Such a result would be expected, for example, if both nuclei were created terrestrially over geologic time in the ratio of their activities, in this case in a production ratio $p(^{92}\mathrm{Nb})/p(^{94}\mathrm{Nb}) = 0.18 \pm 0.11$. We suggest that nuclear reactions following the capture of stopped cosmic-ray muons is the likely source, and we will show that both the magnitude of the activities and their relative strengths are consistent with this idea. Similar ideas have been developed by others, most notably to explain Xe anomalies in Te ores[3].

Examination of a chart of stable nuclides shows that ^{94}Nb in this model arises from the reactions ^{94}Mo (μ^-,γ) ^{94}Nb, ^{95}Mo (μ^-,n) ^{94}Nb, ^{96}Mo $(\mu^-,2n)$ ^{94}Nb, ^{97}Mo $(\mu^-,3n)$ ^{94}Nb, ^{98}Mo $(\mu^-,4n)$ ^{94}Nb, and by ^{93}Nb (n,γ) ^{94}Nb, where the neutrons are regarded as arising from muon capture on all species in the Earth's crust. On the other hand, ^{92}Nb would arise from ^{92}Mo (μ^-,γ) ^{92}Nb, ^{94}Mo $(\mu^-,2n)$ ^{92}Nb, and so on. We use a theory of muon capture as developed by Singer[4] in order to evaluate the various branchings following the muon capture. Molybdenum is the major target for direct production of Nb because charged-particle emission is much less probable than neutron emission. The major uncertainty in our results lies not in the theory, but in the unknown ancient history of the crustal rocks from which the Nb bar was purified. A time average of the Nb/Mo abundance ratio in these rocks enters, because only ^{94}Nb activity can be made by neutron capture on Nb whereas both Nb isotopes result from muon capture by Mo. A pure ancient Nb deposit, for example, would therefore contain only ^{94}Nb activity. Furthermore, the number of neutron captures by Nb per stopped muon depends on the chemical composition of the host mineral through its competition for the neutrons it produces. Apt *et al.* could not discover the history of the ores from which the Nb bar was refined, so we have calculated this competition for two idealised samples: (1) chondrite material, which presumably well represents the average heavy element abundances in the earth, and (2) typical terrestrial granites. Following Singer's description, we constructed a computer programme for calculating the distribution of neutron-emission multiplicities following muon capture on each relevant species, taking the surface-emission term to be 16%. Details of this calculation are given in ref. 5. For evaluating the absolute production, we took the muon stopping rate to be 100–150 muons g^{-1} yr^{-1} down to depths of about 10 m water equivalent[6]. If the ores were considerably deeper than this over the time-integrated production of the anomalous activities ($\sim 10^8$ yr for ^{92}Nb and $\sim$ 10^4 yr for ^{94}Nb), the absolute activities would be considerably reduced.

Table 1 shows major features of our calculation. We assumed the so-called Z-law[7]—that the atomic capture of muons in a mixture is proportional to the nuclear charge Z of the competing atoms—enabling us to calculate in a given composition the fraction of stopping muons captured by each element. In Table 1, $\langle Z \rangle$ is the average charge for each of the two background compositions. The average number $\langle \nu \rangle$ of neutrons emitted per muon stopped is also calculated, as is the average neutron-capture cross section $\langle \sigma \rangle$ of the nuclei of each composition. These allow us to calculate the fraction of the neutrons captured by ^{93}Nb, taking its thermal cross section[8] to be $\sigma = 1.15$ b. The compositional differences are dominated by the chondritic iron being much greater than granitic. The remaining entries in Table 1 show our calculated results for the numbers of each reacrtion occuring per muon stopped in that sample. For the chondritic composition this process is well defined, but for the granitic case we have to assume values for the Mo/Si and Mo/Nb abundance ratios in the rock. We take the latter ratio to be the chondritic value Mo/Nb = 2.2, an assumption that affects the calculated ^{92}Nb/^{94}Nb production ratio. The very small ratio, Mo/Si, does not affect the ^{92}Nb/^{94}Nb production ratio as long as Mo/Nb is held at 2.2; therefore we take it also to be chondritic Mo/Si = 2.5×10^{-6}. Within these assumptions, which are easily scaled to other choices, Table 1 lists the production resulting from each stopped muon in the sample. If the granitic Mo/Si differs from 2.5×10^{-6}, the calculated entry for μ^-–capture reactions scales proportionately, and if Nb/Si differs from 1.15×10^{-6}, the calculated entry for ^{93}Nb (n, γ) scales proportionately. Therefore, the granitic entries can be used more generally than for only the specific trace-element concentrations used in their calculation.

Table 1 ^{92,94}Nb production per stopped muon

	Granitic	Chondritic
$\langle Z \rangle$ matrix	10.4	11.9
$\langle \nu \rangle$, neutrons per muon	0.43	0.54
$\langle \sigma (n, \gamma) \rangle$ matrix(b)	0.16	0.39
^{97}Mo (μ^-, 5n) ^{92}Nb	2.3×10^{-9}	1.4×10^{-9}
^{96}Mo (μ^-, 4n) ^{92}Nb	1.7×10^{-8}	9.9×10^{-9}
^{95}Mo (μ^-, 3n) ^{92}Nb	3.3×10^{-8}	1.9×10^{-8}
^{94}Mo (μ^-, 2n) ^{92}Nb	5.8×10^{-8}	3.3×10^{-8}
^{92}Mo (μ^-, γ) ^{92}Nb	4.7×10^{-8}	2.7×10^{-8}
Total ^{92}Nb per μ^-	1.6×10^{-7}	9.0×10^{-8}
^{98}Mo (μ^-, 4n) ^{94}Nb	2.8×10^{-8}	1.6×10^{-8}
^{97}Mo (μ^-, 3n) ^{94}Nb	1.9×10^{-8}	1.1×10^{-8}
^{96}Mo (μ^-, 2n) ^{94}Nb	1.0×10^{-7}	6.0×10^{-8}
^{95}Mo (μ^-, n) ^{94}Nb	1.3×10^{-7}	7.6×10^{-8}
^{94}Mo (μ^-, γ) ^{94}Nb	2.4×10^{-8}	1.4×10^{-8}
^{93}Nb (n, γ) ^{94}Nb	7.8×10^{-7}	2.7×10^{-7}
Total ^{94}Nb per μ^-	1.1×10^{-6}	4.5×10^{-7}
^{92}Nb/^{94}Nb per μ^-	0.14	0.20

The calculated production ratios per captured muon, $p(^{92}\text{Nb})/p(^{94}\text{Nb}) = 0.14$ for granite and 0.20 for chondritic matter, bracket the steady-state production ratio 0.18 ± 0.11 needed to match the ratio of observed activities. Furthermore, the absolute concentrations are also about right. The observation[1] ^{92}Nb/^{93}Nb = 1.2×10^{-12} translates in our model granite to 8.7×10^3 atoms ^{92}Nb (g granite)$^{-1}$. If interpreted as a steady-state concentration, this number must equal the product 1.6×10^{-7} τ (^{92}Nb) λ_μ, where the coefficient comes from Table 1 and λ_μ is the muon stopping rate per gram of granite per year. The required stopping rate, $\lambda_\mu = 222$ g^{-1} yr^{-1}, is about 50% greater than the value[6] at a depth several metres water equivalent under the earth. Virtually the same stopping rate would be needed in chondritic material. The rough agreement in production ratio and in absolute yield attests strongly to the probable correctness of this explanation. Neutrons from other sources near the Earth's surface or from (α,n) reactions within the crust[1] may synthesise ^{94}Nb from ^{93}Nb, but the associated ^{92}Nb activity suggests that muons are a major source.

Major uncertainties exist in the history of the ore, of course, and these cloud firm conclusions. If deep material has risen to within 10 m from the surface, each activity within will have increased in time as muon reactions built the activity to saturation; however, since saturation requires roughly 10^8 yr for ^{92}Nb and only 10^4 yr for ^{94}Nb, time dependent effects can alter the activity ratio. There is also a major question of fractionation of Mo from Nb during the growth of these activities and the geologic processes leading to the mining sites. In pure Nb the ^{92}Nb/^{94}Nb activity ratio should be very small, whereas Table 1 shows in pure Mo it should be about 0.5. In principle, such variations could yield interesting evidence of geologic history, because the activities of these two isotopes should differ in Nb samples purified from ores having different geologic histories. Searches for these activities in old molybdenum ores should prove interesting. It would also prove helpful if a Mo bar could be exposed to a sufficient flux of stopping muons to experimentally determine the ^{92}Nb and ^{94}Nb activities, for then our calculated sums of branching ratios could be replaced by experimental numbers, greatly improving the accuracy.

The idea that the ^{92}Nb could be a remnant of galactic p-process nucleosynthesis is of course an attractive hope. But, we find little grounds for optimism from p-process systematics. Most p-process nuclei seem to be produced as proton-rich progenitors, in which case ^{92}Nb is shielded by ^{92}Mo. The ^{92}Nb is similar to other heavy odd–odd nuclei ^{138}La and ^{180}Ta, both of which are very rare and may be taken as empirical guides to p-process nucleosynthesis in such cases. Their abundances are 10^{-1} to 10^{-2} of characteristic p-abundances in their neighbourhood. Taking the same estimate for ^{92}Nb leads to a total ever produced $^{92}\text{Nb}_{\text{total}} \sim 0.02$ to 0.002 per 10^6 Si, where we have estimated the average $N_p = 0.2$ by averaging ^{84}Sr, ^{94}Mo and ^{96}Ru, excluding ^{92}Mo which is known to be anomalously large. A photodisintegration-flow p-process calculation[9] has recently yielded $^{92}\text{Nb}_{\text{total}} = 0.001$, an even lower estimate of total production. Our models of continuous nucleosynthesis suggest that about 2% of the total ^{92}Nb would have survived to the early Solar System; therefore $^{92}\text{Nb}_0 = 4 \times 10^{-4}$ to 4×10^{-5} per 10^6 Si, or $(^{92}\text{Nb}/^{93}\text{Nb})_0 = 3 \times 10^{-4}$ to 3×10^{-5}. This ratio could be enough to account for today's activity if $t_{1/2} \geqslant 1.7 \times 10^8$ yr, so again, one awaits a better halflife measurement. Even then, the expected amount is perhaps too small to expect to find δ (^{92}Zr) anomalies due to extinct ^{92}Nb[2].

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Particulate organic carbon in Atlantic surface waters

PARTICULATE organic matter (POM) in the oceans has a vital role in marine ecology as it is the major food source for living organisms in surface and deep waters. Nearly all of the suspended organic matter in the oceans originates from phytoplankton productivity in the euphotic layers and is comprised of living plankton and dead organisms (detritus). Because of the importance in the marine food web of suspended organic phases in seawater several investigations on their distribution and composition have been carried out[1–3].

Most of the available data, however, are based on sampling at a single location and hence the magnitude of latitudinal variation in the POM concentrations are not well established. Chester and Stoner[4] have reported extensive measurements on the concentration of particulate organic carbon (POC) and particulate nitrogen (PN) in surface waters of the eastern Atlantic and Indian oceans. We present here results on the distribution of POC in the open ocean surface waters of the

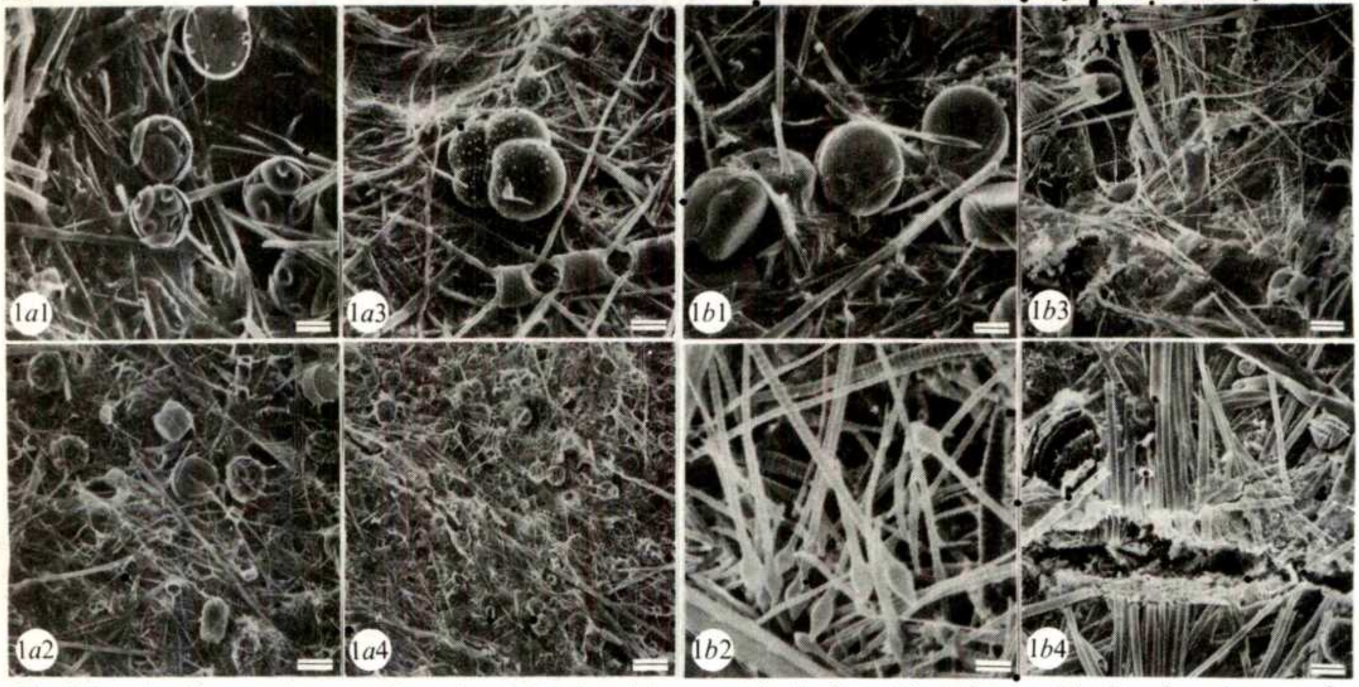

Fig. 1 Scanning electron microscope photomicrographs of particles trapped in the filters. Most of the particles in *a*, from the coast of Iceland and the Norwegian Sea, are calcareous (coccolithophoridas and forams); the large circular particle on the top and the rod-like particles are siliceous. In *b*, from the Antarctic and the equatorial waters, the particles are mostly siliceous. Scale bars: *a*1, 5 µm; *a*2, 10 µm; *a*3, 10 µm; *a*4, 20 µm; *b*1, 5 µm; *b*2, 5 µm; *b*3, 20 µm; *b*4, 20 µm.

Atlantic, between 72°N and 61°S; 60°W and 20°E, and its interrelation with primary productivity.

The samples were collected during the GEOSECS Atlantic expedition (July 1972–April 1973) by filtering large volumes (2,000–40,000 l.) of surface seawater while cruising between stations, usually 3° apart. Thus, the particles were trapped from representative samples of surface waters over hundreds of km. We used a cotton fibre filter with almost quantitative retention efficiency for particles > 5 µm and 60% for particles in the 0.5–1.2-µm range. The filter characteristics and filtration techniques are discussed in detail elsewhere[5]. The filters were rinsed with sufficient quantities of distilled water to remove sea salts, dried on board the ships at 90–100 °C, packed carefully and sent in poly-bags to shore-based laboratory for analysis. Figure 1 shows some of the trapped biogenic particles on the filter. The weight of total particulate matter (TPM) (the weight difference in the filter before and after the experiment) trapped per filter was 0.1–3.0 g. The samples were ashed at 400–425 °C, and the weight loss on ashing was attributed to POM. Particulate organic carbon content of the samples was calculated by assuming that it constitutes 50% of POM (ref. 7). The calculated particulate organic carbon is designated as CPOC henceforth.

The TPM and CPOC concentrations in surface waters ranged between about 10–600 and 2–170 µg kg^{-1}, respectively, and are strongly interrelated (Fig. 2). The latitudinal distributions of TPM and CPOC concentrations (Fig. 3) are similar, with pronounced maxima near subpolar regions (~60°N and 60°S) and low concentrations in the tropics and mid-latitudes (30°N–40°S).

The relation between TPM and CPOC for the surface Atlantic suspended phases in different latitudinal belts are given in Table 1. The mean TPM/CPOC for the Atlantic (72°N–61°S; 60°W–20°E) is 3.94, corresponding to a mean CPOC content of ~25%, that is, ~50% POM. The remaining ~50% of TPM is composed of biogenic skeletal remains, ($CaCO_3$ and opal) and terrestrial materials (aluminosilicates, quartz). The TPM/CPOC ratios exhibit geographical variations (Table 1, Fig. 2). For samples from northern subpolar regions (45–72°N) the mean ratio is (3.57±0.38) compared to a value of (4.8±0.7) for samples from 45–61°S. The samples from the 45°N–45°S region have the lowest TPM/CPOC ratio, 3.15±0.18. The observed TPM/CPOC ratios are in good agreement with results of Chester and Stoner[4]. The differences in the TPM/CPOC ratios in various regions seem to be due to variations in the elemental composition of biogenic populations

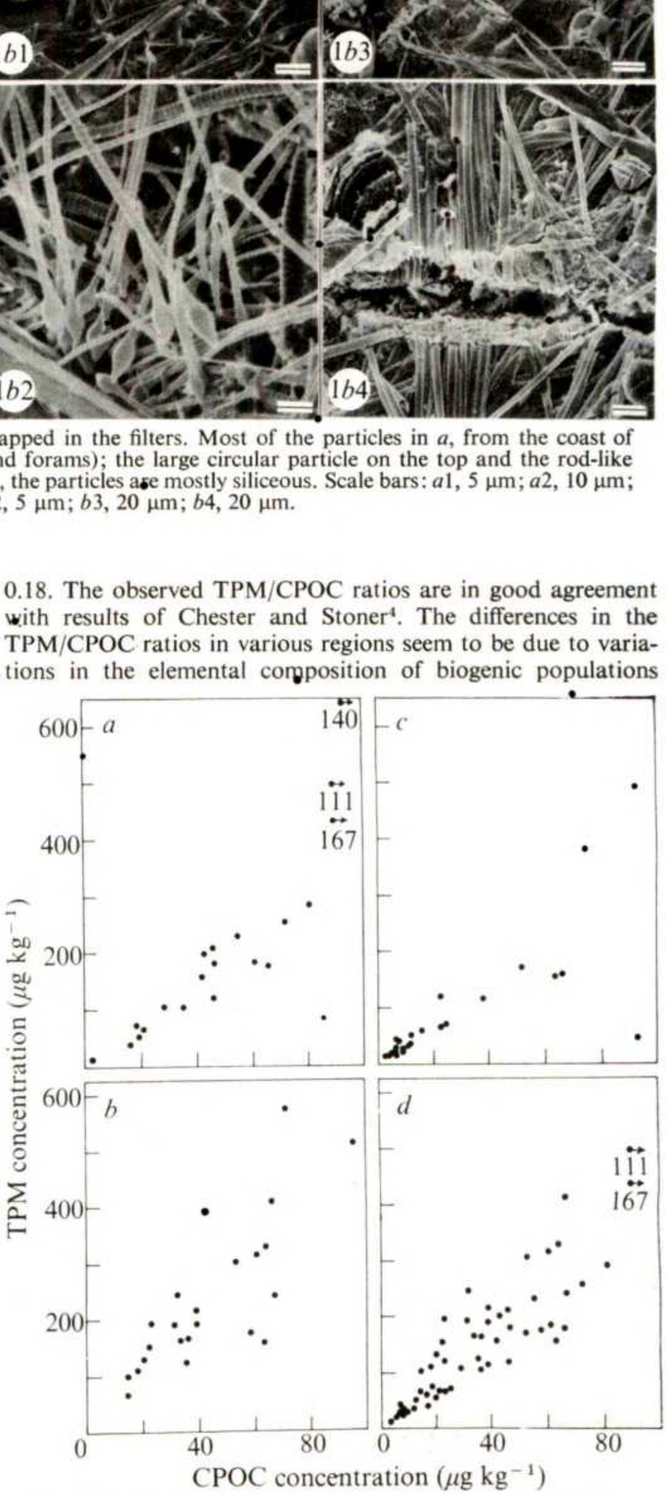

Fig. 2 Correlation diagram for the TPM–CPOC concentrations for various regions of the Atlantic surface waters. The linear correlation between TPM and CPOC is evident. Note that CPOC constitutes about 20% of TPM in samples from Antarctic circumpolar region compared to ~30% for rest of the Atlantic. Only samples having ⩾ 200 mg weight loss during ashing were taken to avoid possible uncertainties due to weightloss of filter during filtration. *a*, 72°–45°N; *b*, 45°–61°S; *c*, 45°N–44°S; *d*, 72°N–61°S.

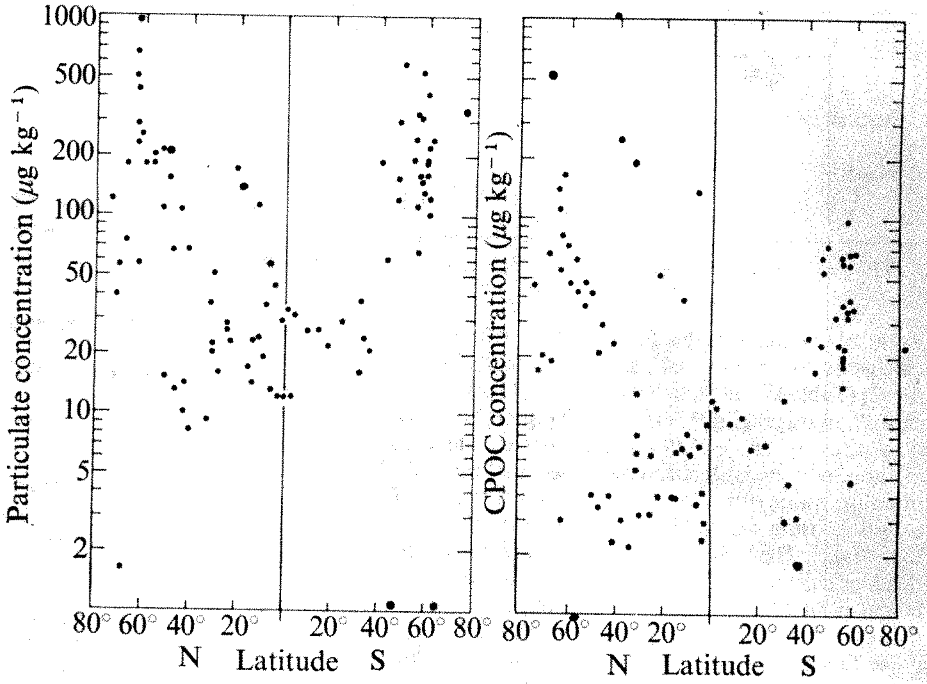

Fig. 3 Latitudinal distribution (72°N–61°S) of TPM and CPOC in Atlantic surface waters. The distribution patterns for both TPM and CPOC are almost identical, with pronounced maxima in the subpolar regions due to high organic productivity. Low concentrations have been observed for waters between 30°N and 30°S.

occurring in these bands. Our detailed analyses[8] indicate that siliceous tests dominate in the Antarctic circumpolar waters (40°–60°S) whereas calcareous tests are relatively more abundant in the North Atlantic (Fig. 4).

The regional differences in the CPOC concentrations of surface waters can be roughly correlated with nutrient supply due to upwelling and phytoplankton activity. In the North Atlantic, high CPOC concentrations were observed in the Denmark Straits and off the coast of Iceland, where upwelling of nutrient-rich waters caused by passage of strong currents over the Iceland–Faroes Ridge occurs, and in the Canary Islands, where cool nutrient-rich water ascends to the surface due to off-shore wind action[9]. Similarly, CPOC concentrations are high south of the Antartic Convergence zone, where very high primary productivity has been observed due to upwelling of circumpolar waters. Lowest CPOC concentrations are in the western Atlantic, between 30°N and 30°S. Our results have the following implications: (1) Organic biological matter constitutes ~50% by weight of the total suspended matter in the Atlantic Ocean. (2) Organic carbon in sediments constitutes only ~0.3% compared to a value of ~25% in surface particulates. This suggests that most of the organic carbon produced in surface waters dissolves within ocean water. (3) The mean residence time of particulate organic carbon within the top (100–200-m) layer against consumption by dissolution and grazing is deduced to be about 5–10 d. This is based on the ratio of the standing crop of particulate carbon (from our measurements in surface waters) and its primary production rate[12]. The standing crop of particulate organic carbon, $\Sigma\mathrm{POC} = C_s L$, where C_s is the surface concentration and L is the mean concentration depth, the depth over which the concentration decreases by a factor of e. The

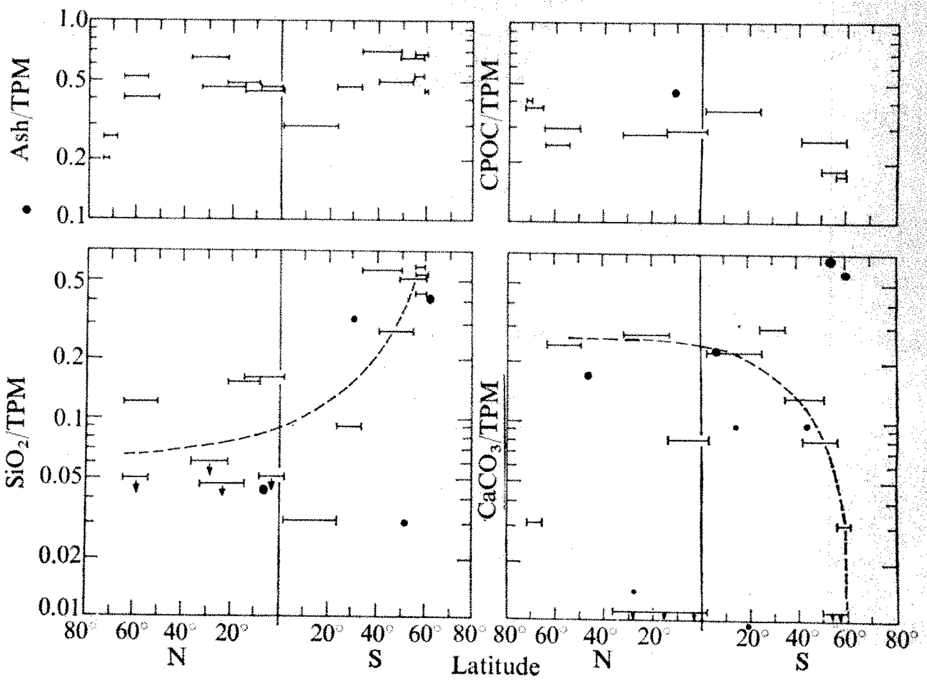

Fig. 4 Latitudinal variation of the ash, CPOC, CaCO$_3$ and SiO$_2$ contents of TPM from surface Atlantic waters. The CaCO$_3$ is relatively more abundant in the northern latitudes, whereas samples from ~60°S contain mainly siliceous organisms.

Table 1 TPM, CPOC interrelation for surface Atlantic waters in various latitudinal belts

Latitudinal belt	No. of samples	CPOC concentration ($\mu g\ kg^{-1}$) Range	Mean	TPM–CPOC relation* ($m \pm \Delta m$)	C	Correlation coefficient for TPM–CPOC relation
72°N–45°N	19	17–167	59	(3.57 ± 0.38)	0.9	0.92
45°N–45°S	28	3–52.5	12	(3.15 ± 0.18)	3.8	0.96
45°S–61°S	22	14.5–96	44	(4.8 ± 0.7)	20.3	0.81
72°N–61°S	69	3–167	35	(3.94 ± 0.25)	10.2	0.89

*TPM = $(m \pm \Delta m) \cdot$ CPOC+C, where TPM and CPOC are their concentrations in $\mu g\ kg^{-1}$ and C is a constant.

value of ΣPOC for the (45°N–45°S) belt in the Atlantic is calculated to be about 60 $\mu g\ cm^{-2}$ using a value of 12 $\mu g\ kg^{-1}$ for C_s (Table 1) and 50 m for L (refs 12, 13). Primary production rate, P, in this region[14] is about 100 $mg\ m^{-2}\ d^{-1}$. The consumption time, τ_c ($\tau_c = \Sigma POC/P$) of particulate organic carbon in these regions is thus estimated to be about 6 d. The corresponding value for the Atlantic surface waters (72°N–61°S) is ~9 d, using $C_s = 35\ \mu g\ kg^{-1}$, $L = 50$ m and $P = 190$ mg C $m^{-2}\ d^{-1}$. The estimated value for τ_c refers to both living and dead organisms. The mean survival time for living organisms is much smaller, about 1–2 d, as the ratio[1] of living phytoplankton to detritus is approximately 1:6.

(4) The average SiO_2/CPOC molar ratio in particles from the circumpolar waters (~60°S) is about 0.4. Thus in the Antarctic polar front, biological productivity (primarily siliceous organisms) converts 0.4 mol of soluble silica into suspended phases per mol of carbon. Biogenic silica constitutes more than 90% of the total particulate silica at 50–60°S.

(5) The non-uniform distribution of the TPM and CPOC concentrations suggests that horizontal mixing due to surface currents is not effective in homogenising the particle concentrations; the sinking rates of these suspended phases, therefore, must be faster than the surface current velocities. As most of the CPOC (~95%) dissolves in the water column before being deposited as sediment, the trace elements associated with particulate organic carbon phases are also expected to be injected in seawater in dissolved form. If so, the dissolution flux of elements could also have geographical dependence governed by primary productivity in various latitudinal bands. The dissolution flux of elements associated with the hard skeletal part of the organisms (for example, $CaCO_3$ and opal) will similarly show geographical variation, as the $CaCO_3$/TPM and SiO_2/TPM ratios vary considerably in surface waters.

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Nickel in komatiites

THE principle of partitioning of trace elements between magma and crystalline phases is an extremely useful tool in deciphering the origin and evolution of magmas. With our present knowledge of partition coefficients calculations usually assume them to be constant. The nickel contents of suites of komatiitic lavas from South Africa, the evolution of which is controlled by olivine fractionation, suggest that the partition coefficient for nickel between olivine and liquid is not constant, but increases from <3 for liquids with >20% MgO and >1,000 p.p.m. Ni to 11 for liquids with 10% MgO and 200 p.p.m. Ni. There are two fundamentally different views on the origin of basic magmas. O'Hara[1] proposed that the mantle melts to produce a picritic liquid which undergoes extensive olivine fractionation prior to eruption. The alternative model, summarised by Ringwood[2], assumes that basic magma is a direct product of melting and undergoes no fractionation. Ringwood's rejection of the fractionation hypothesis is based largely on calculations of the Ni content of magmas[2]. All his calculations assume a constant partition coefficient for Ni between olivine and liquid in the range 12–15. Several recent investigations concluded that K_D is not constant but that it decreases (1) with increasing temperature[3,4]; (2) with increasing MgO content in the liquid[5]; (3) with increasing Ni content in the liquid[6], and (4) as crystallising olivines become more forsteritic[7]. These effects have been reviewed in detail elsewhere[8] but the net effect is that for a liquid, rich in MgO and Ni, crystallising a forsteritic olivine at high temperature, K_D may be significantly lower than the figure currently accepted. Komatiitic lavas fall perfectly into this category of liquid as they have high MgO and Ni contents and it has been shown experimentally[9] that they crystallise very magnesian

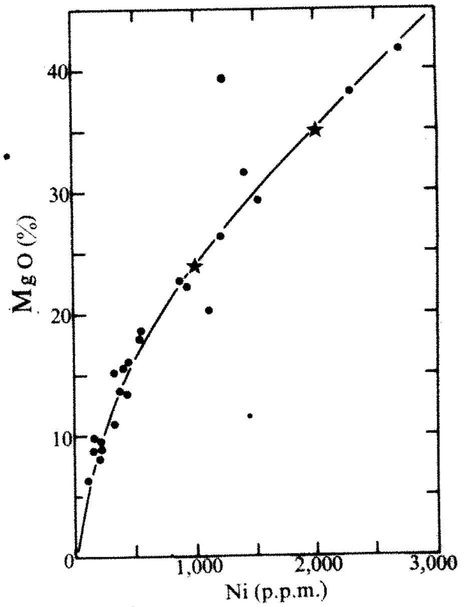

Fig. 1 Plot of Ni against MgO contents of komatiites analysed in Table 1. Stars denote proposed parental magma compositions.

olivines at temperatures well above the liquidus for normal basalts. Most of the studies which have indicated low K_D values have involved experimental work on synthetic samples[4–7], some of which[5–7] were iron-free and hence, although they represent an extremely important step towards understanding partition coefficients, quantitatively the K_D values may be suspect. We have analysed the Ni contents of komatiites with a wide range of compositions to determine the partition coefficient for these seldom-erupted lavas to test more rigorously the constraints imposed by Ni values on the fractionation model for basic magmas.

Table 1 MgO and Ni in komatiites and calculated degrees of fractionation

Sample no.	MgO (% by weight)	Ni (p.p.m.)	Olivine removed* (%) Model 1	Model 2
S3	18.46	529	51	17
S7	15.75	407	55	23
S11	15.96	424	55	23
S16	13.69	383	58	27
S28	17.93	512	52	18
OV3	13.57	423	56	27
OV4	11.09	348	58	31
OV5	9.76	169	60	34
SG3	20.38	1102	48	11
SG80	22.28	935	45	6
VB1	15.45	337	56	24
VB4	9.83	179	59	34
V11	6.37	107	63	38
40J	8.08	205	62	36
53J	31.64	1407	17	C
87J	22.98	862	43	4
88J	26.58	1203	35	C
R16	41.58	2716	C	C
AUS	29.36	1633	25	C
VU32a	38.13	2298	C	C
LV4	9.40	210	60	34
LV7	8.50	205	61	35

C indicates MgO content is higher than in the theoretical parental magma used in the calculations and hence is regarded as a cumulus-enriched rock. S3–S28 from Anhaeusser (unpublished), OV3–OV5 from ref. 11, SG3–LV7 from ref. 10.

*F, proportion of liquid remaining is (100—% olivine removed)/100.

The geological relations for most of the komatiites chosen for analysis (from the Barberton Mountainland in South Africa) have been described elsewhere[10]. All the samples are demonstrably lavas; as indicated by pillow structures, vesiculation or spinifex textures; except sample 53J which is the chilled margin of a dyke. Their MgO and Ni contents are given in Table 1 (complete major element analyses have been published for most samples (refs 10, 11 and C. R. Anhaeusser, unpublished)). The values are calculated on an H_2O^+-free basis (Fig. 1), and a very close correlation between Ni and MgO is apparent. It has been suggested[10,12] that these samples are related by olivine fractionation, and hence for these suites of lavas we have an opportunity to study the variation in Ni content produced by the removal of olivine.

Green *et al.*[9] have suggested that komatiitic samples containing 35% MgO represent liquids while McIver[12] and Cawthorn and McIver[13] believe such compositions to be cumulus-enriched in olivine and that the true liquids contain significantly less than this. Here we interpret the data using two hypothetical parental magmas, one with 35% and the other with 24% MgO. Figure 1 suggests that such liquids contain 2,000 p.p.m. and 1,000 p.p.m. Ni respectively. The olivine composition which crystallises from these magmas is approximately Fo_{93} which contains 51% MgO (ref. 9). Starting with parental magmas containing 35% and 24% MgO it is possible to calculate the degree of olivine fractionation required to produce the MgO contents of residual liquids with the MgO values given in Table 1. These calculated degrees of fractionation are also listed in Table 1: models 1 and 2 referring to parental magmas with 35% and 24% MgO respectively.

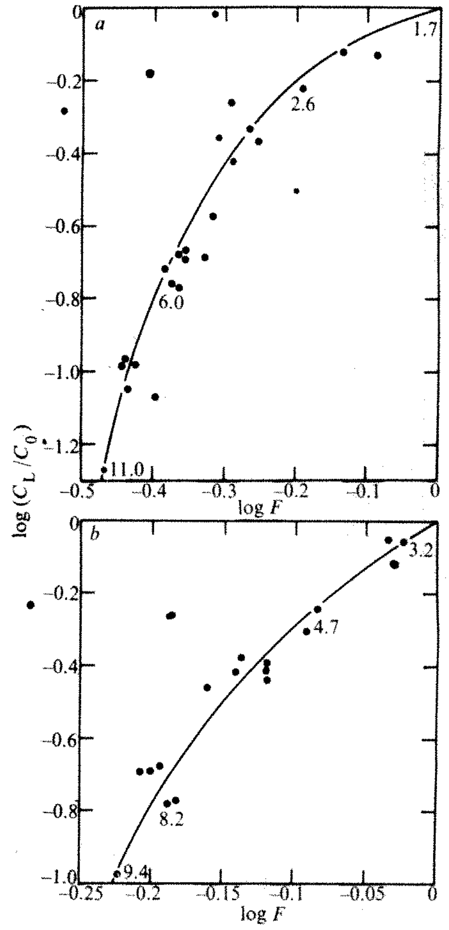

Fig. 2 Plot of log (C_L/C_0) against log F. Slope of graph is $K_D - 1$ (as shown in text) and values for K_D are given at various points. *a*, Data for model 1 which assumes the parental magma contains 35% MgO and 2000 p.p.m. Ni. *b*, Model 2 for a parental magma with 24% MgO and 1,000 p.p.m. Ni.

The Rayleigh fractionation law states $C_L/C_0 = F^{K_D - 1}$ where C_0 is the trace element content of the parental magma, C_L that of the differentiated magma; F the proportion of differentiated magma produced, and K_D the partition coefficient. This may be rewritten as $\log(C_L/C_0) = (K_D - 1)\log F$.

Assuming 2000 p.p.m. (model 1) and 1000 p.p.m. Ni (model 2) for C_0 and having calculated the value for F from Table 1 we can plot a graph of log (C_L/C_0) against log F (see Fig. 2) and this will give a slope of $K_D - 1$. It is readily apparent from Fig. 2 that the graphs are not linear and hence the partition coefficient cannot be constant. The slope of the curves has been calculated at various points and the value for K_D, derived from the slopes, included in Fig. 2. Considering the model proposed by Green *et al.*[9], the initial slope of the curve is 0.7 and hence K_D is 1.7. (It is interesting to note that the experimentally determined K_D of Green *et al.*[9] is only 2.1 and hence agreement between experiment and these calculations on natural samples is extremely good.) At the extreme end of differentiation considered here, for liquids containing 6.37% MgO, the K_D as calculated from the slope of the graph is 11. Alternatively, if we consider model 2 the initial partition coefficient for liquids containing 24% MgO is 3.2 and has a maximum value of 9.4. Pyroxenes may begin to crystallise from magmas with low MgO contents. Hence, it may be that some of the most differentiated liquids of this study are not the product of fractionation of olivine alone. The K_D values determined for these low MgO liquids may not therefore apply to olivine : liquid partitioning, but this will not qualitatively alter the arguments presented here.

The values for the partition coefficient for low MgO-liquids (<8% MgO) are fairly close to those predicted for basic magma[14]. Hence, for such compositions the partition coefficients determined for these komatiites and other magmas appear compatible. For the MgO-rich compositions, however, the values are very much lower.

With reference to the debate between Ringwood and O'Hara mentioned earlier we conclude for the partition coefficients calculated here that: (1) the primary picritic magma envisaged by O'Hara[1] may contain very much higher nickel contents than suggested by Ringwood[2] because the partition coefficient is much lower. And (2) the rate at which nickel will be extracted from this parental magma by olivine fractionation will be slower than anticipated by Ringwood[2]. Specifically, it is apparent from Table 1 that an initial magma containing 24% MgO and 1000 p.p.m. nickel may undergo 40% olivine fractionation to produce a residual liquid with 6% MgO and 100 p.p.m. nickel—values perfectly compatible with those of typical basic magmas. O'Hara[1] does not quantify his melting and crystallisation models, but these figures presented above suggest that the variation in partition coefficients makes his models qualitatively more tenable.

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Rhythm perception in early infancy

THE chief characteristic of rhythm perception is the subjective grouping of objectively separate events. In a rhythmic sequence of identical tone-bursts, adults do not perceive the repetition of a single sound, but a recurring configuration which has temporal form. Such a sequence is organised according to the Gestalt law of proximity[1,2]. Organisation of temporal form has never been studied systematically in pre-verbal infants. We present here results suggesting a precocious achievement of this function and contrasting with previous research[3] which failed to demonstrate organisation of spatial form by babies in accordance with the proximity law.

We adopted an habituation paradigm in which infants received auditory reinforcement contingent upon their visual fixation on a simple patterned-figure: a black outline-square on a white background. The infants themselves initiated the presentation of sound by looking at the figure and terminated it by looking away. After several such fixations, the auditory reinforcement was changed from one sound sequence to another. Any resulting increase in time of fixation on the visual stimulus was an index of discrimination.

Sequences of tone-bursts were presented by a loudspeaker placed directly behind the stimulus figure at a distance of 40 cm from the infant. The tone-bursts, 40 ms in duration, were derived from a tape-recording of a consonant chord produced by an electronic organ, with 270 and 1,620 Hz as dominant frequencies; intensity was 63 dB, about 25 dB above the ambient noise level. The stimulus figure subtended a visual angle of 8° and was positioned 20° to the left of midline. Fixation, as assessed by the corneal reflection technique[4], was observed from directly in front of the infant, through a small hole in the screen containing the figure. The infant was judged to be fixating when, his eyes being turned to the left, the stimulus reflection was clearly observable over the right of the pupillary opening. Duration of fixation, to the nearest tenth of a second, was recorded manually by a switch which simultaneously operated sound-emission and an event-recorder. In previous research, a high inter-observer reliability has been obtained for this measure[5].

The first experiment was designed to demonstrate discrimination between the rapid, quite different sequences, S1 and S2, shown in Fig. 1. A regular pulse of tone-bursts, S1, was changed to an irregular sequence, S2. The two sequences had the same mean density of sound. We tested two samples, each of 10 infants, with mean ages of 71 d (s.d. 12) and 107 d (s.d. 6) respectively. Three fixations without sound reinforcement were followed by 12 fixations reinforced by S1 and then three fixations reinforced by S2. Median fixation times are shown in Fig. 2*a*. A significant increase in fixation time followed the change in reinforcement ($F = 10.37$, $P < 0.005$), indicating discrimination of the two sequences. No main or interaction effect was associated with age.

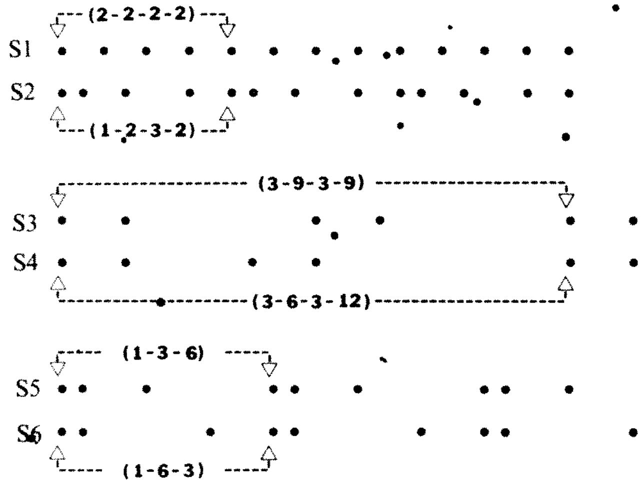

Fig. 1 Sound sequences. Each dot represents a 40-ms tone-burst. The shortest onset-to-onset interval was calibrated as 97 ms. All intervals were multiples of this unit. Their relative magnitudes in each sequence are given in brackets. Arrows delimit the period of each sequence. For example, the period of S2 is a succession of 1, 2, 3 and 2 time units (that is 97, 194, 291 and 194 ms). A sequence started always at the beginning of its period, and terminated when visual fixation ended (experiment 1) or after completion of the period in progress at that instant (experiments 2 and 3). Scale bar represents 1 s.

In a second experiment, we studied discrimination of slower rhythms, using the sequences S3 and S4 depicted in Fig. 1. The visual fixations of 10 infants (mean age 69 d; s.d. 18) were contingently reinforced nine times by S3 and then three times by S4. Fixation time increased ($t = 3.72$, $P < 0.005$) when S4 replaced S3 (Fig. 2*a*). Thus, the 6-unit intervals of S4 were discriminated from the 12-unit intervals of S4 or from the 9-unit intervals of S3; alternatively, a new ratio of intervals was detected in S4. In any case, we conclude that infants are able to perceive intervals of six units (582 ms) as subjective links between sounds.

The aim of the third experiment was to examine perception of temporal form in sequences differing only in the order of their intervals. In the preceding experiment, discrimination might be attributed solely to the detection of a new interval. In experiment 3, however, the same intervals were used in both sequences, and the minimum segment for distinguishing between them was composed of a pair of successive intervals. Thus, the grouping of three consecutive sounds into a perceptual configuration was mandatory if discrimination was to occur. The longest interval appearing in S5 and S6 (Fig. 1) had already been shown to be

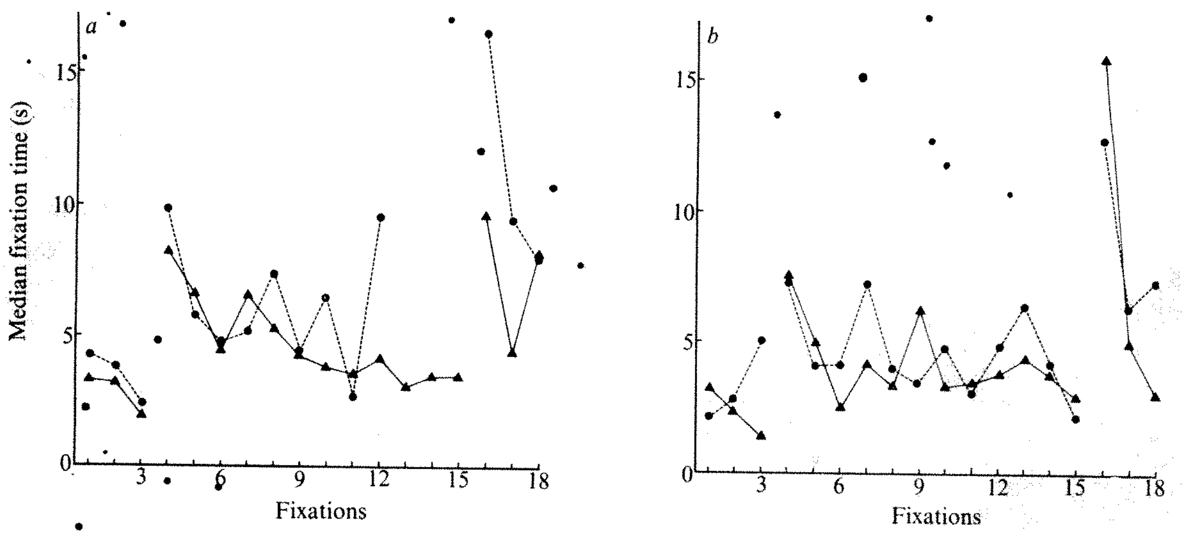

Fig. 2 *a*, Median time of fixation in experiments 1 and 2. In experiment 1 (▲), there was no sound reinforcement for fixations 1–3. S1 was presented contingently with fixations 4–15, and S2 with fixations 16–18. In experiment 2 (●), there was no sound reinforcement for fixations 1–3. S3 was presented contingently with fixations 4–12, and S4 with the final three fixations. The duration of the first fixation after change of reinforcement was compared with the mean duration for the preceding three fixations. *b*, Median time of fixation in experiment 3. No sound reinforcement was associated with fixations 1–3. For Group 1 (●), S5 was presented contingently with fixations 4–15 and S6 with fixations 16–18. For Group 2 (▲), the order of sequence presentation was reversed. The duration of fixation 16 was compared with the mean duration for fixations 13, 14 and 15.

perceptible in experiment 2. Therefore, within each reinforcement, all the sounds may have been linked in a temporal continuum. The first interval in each sequence was the same in order to ensure that discrimination was not based solely on a difference in this first interval, without further grouping. To adults, the sequences sound like recurring groups of three pips, the groups (. .   .) in S5 being perceived as the inverse of the groups (.   . .) in S6. In all emissions of S6, however, the first and final groups were incomplete (see Fig. 1). We tested two samples, each of 10 babies. Group 1 (mean age 69 d; s.d. 12) was tested in the order S5 followed by S6, and Group 2 (mean age 70 d; s.d. 18) was tested in the reverse order. Median fixation times are shown in Fig. 2*b*. An increase in fixation time followed the change in sequence ($F = 11.81$, $P < 0.005$); order of testing was associated with no reliable main effect or interaction. Since the differentiating of these sequences entails the grouping of three successive sounds, we infer that they were perceived as recurring 'rhythmic groups' organised in temporal form according to the Gestalt law of proximity. In addition, it may be noted that, following the first half-second, the rhythms can be distinguished only by the order of the within-group intervals. The magnitude of the novelty response suggests that, rather than perceiving only a single, very brief change, the infants have also noted the repetitive difference in within-group structure.

A succession of several sounds was apprehended by babies as a psychological unit. This phenomenon of temporal grouping is, of course, not peculiar to rhythm perception. Language comprehension, for example, requires a similar process, since a semantic unit must be grasped as a whole, despite its sequential character. In the results reported here, perceived structure rests on a purely temporal variable: the relative values of the intervals between sounds. These results indicate that skills of temporal analysis and synthesis, prerequisites for operating with the sequential dimension of language, are present well before the stage of speech, and suggest that the early experience of time is more complex than is generally assumed.

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The whalebill is a stork

THE avian order Ciconiiformes contains a heterogeneous assemblage of long legged, long necked birds which have traditionally been classified together because of superficial resemblance. The order usually contains the storks, ibises, herons and two rather aberrant birds, the whalebill (*Balaeniceps rex*) and the hammerkop (*Scopus umbretta*), which are placed in monotypic families, the Balaencipitidae and Scopidae, respectively. The evolutionary relationships among the members of the Ciconiiformes and between this order and other living birds are little understood. Using an analysis of the morphology of the stapes (the single avian ear ossicle)[1,2], I have obtained evidence for the evolutionary affinity of the whalebill with the storks, which now seem to form a monophyletic assemblage.

The whalebill, whalehead or shoebill (Aves: *Balaeniceps rex*) is a strange, storklike bird with an enormous swollen head and a hooked beak. It is restricted to marshes and riverine situations in eastern Africa from the southern Sudan to Zambia. It is largely nocturnal, and feeds on various vertebrates including frogs, snakes and even lungfishes, but also molluscs and carrion. It has many similarities with the true storks (including bill clattering behaviour), but its systematic position has always been uncertain. Recently it has been thought to have more similarities with the pelecaniform birds (order Pelecaniformes), while the resemblance to storks was thought to be superficial[3]. The characters used, however, were not analysed on the basis of primitive and derived states.

Study of the morphology of the stapes, or columella, makes possible a reassessment of the evolutionary affinities of the whalebill and the storks. The stapes is of great evolutionary significance as sequences of primitive to derived characters can be established beyond reasonable doubt. The typical avian stapes is reptilian, with a flat footplate and a straight bony shaft emanating from the centre of the footplate. Several avian groups, however, have unique, derived stapedial morphologies. I have assumed that: (1) the stapes found in most birds (most of the non-passerines and the suborder Passeres of the Passeriformes, which I now restrict to a separate order Passeriformes[1,2]) is homologous with the same element in reptiles, and represents a retained primitive condition, and (2) where pockets of derived stapedial morphologies occur, they indicate evolutionary relationships, unless there are compelling reasons to assume that convergent evolution has occurred.

I have examined the stapes of the whalebill (*Balaeniceps*), 23 species of herons (13 genera), 10 species of ibises (8 genera), and 11 species of storks (9 genera), including the small form, *Ciconia abdimii* (75 cm long), to avoid anatomical effects due to allometric growth; most storks are large birds. I have also studied the stapes of the monotypic family Scopidae, which contains the equally enigmatic hammerhead or hammerkop (*Scopus umbretta*), a species also confined to Africa south of the Sahara. All specimens were from the collections of the US National Museum and the University of Michigan.

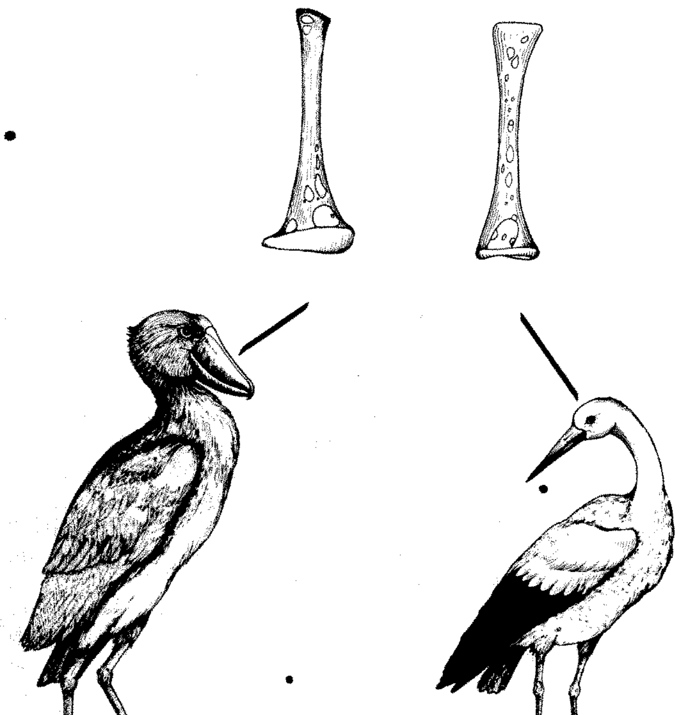

Fig. 1 Left, the whalebill (*Balaeniceps rex*), and right, the white stork (*Ciconia ciconia*), with lateral views of their stapes or columellae above; stapes drawn to the approximate same scale. The proximal ends are lower. Drawn by Ms Melissa Marshall.

I found that all the herons (Ardeidae, including the boat-bill, *Cochlearius cochlarius*), all the ibises (*Threskiornithidae*) and *Scopus* (Scopidae) have the primitive condition of the stapes, thus revealing nothing of their phylogenetic relationships, while all the storks and the whalebill have an identical, derived stapedial morphology. Pelecaniform birds have various stapedial morphologies that will be described in detail later together with those of the groups considered here, but they are quite different, elongate, somewhat curved structures. The stapes of the storks and whalebill (Fig. 1), here termed the stork stapes, is characterised as a broadly tubular structure that enlarges slightly at the footplate region, and exhibits numerous fenestrae along the entire surface, usually with one or several large openings at the base near the footplate. Such a derived stapedial morphology common to the storks and the whalebill is interpreted as compelling evidence that the storks as defined at present are monophyletic and that the whalebill (*Balyaeniceps*) is derived from them. Some authors[4] have made a case (primarily on osteological grounds) that the storks and New World vultures (Cathartidae) are closely allied; however, all of the cathartids possess the primitive stapedial morphology.

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Early viral functions of *in* (ts)-1501 temperature-sensitive SV40 mutant

TEMPERATURE-SENSITIVE (ts) virus mutants have been useful in defining virus–host interactions in SV40 productive and transforming infections. We have isolated an SV40 ts mutant, *in*(ts)-1501, by extracellular mutagenesis, using ultraviolet radiation of wild type (WT) SV40[1]. The mutant, which has properties different from previously described SV40 ts mutants[2,3], is activated from its integrated state in nonpermissive transformed cells at temperatures above 39 °C and is capable of productive infection in permissive cells at elevated (conditional) temperatures[1]. Many properties of the *1501* mutant are analogous to the early ts cI mutants of bacteriophages in which a heat-inactivated protein cannot maintain the stable integration of the prophage in the bacterial chromosome at elevated temperatures[4-8]. This analogy suggested that maintenance of SV40 DNA integration in transformed cells requires an early viral function, that is, before the inception of viral DNA replication. Therefore we have investigated the effect of the *in* gene mutation on two other early virus functions—stimulation of host-cell DNA synthesis and tumour (T)-antigen synthesis.

Ts*A* mutants, the only previously identified mutants mapped in the early region of the SV40 genome[9], show normal induction of host-cell DNA synthesis at 40 °C, but some show a reduced induction at 42.5 °C (ref. 10). We studied stimulation of host-cell DNA synthesis in *1501* ts mutant-infected and WT SV40 strain 777-infected confluent TC7 cells. TC7 is a continuous line of African green monkey kidney cells that is permissive for SV40 productive infection[11]. The results (Table 1) indicate that the *1501* mutant can induce host-cell DNA synthesis at both conditional and nonconditional temperatures.

SV40 T-antigen, a nonstructural virus-coded protein, is expressed in the nuclei of productively and abortively infected and transformed cells. Approximately 30–40% of the SV40 genome is represented by this protein, the molecular weight of which has been estimated to be 70,000–100,000 (refs 12 and 13). The T-antigen is the SV40 *A* gene product, as defined by the ts*A* group of SV40 mutants[13-17]. Although Brugge and Butel[18] found that the synthesis of T-antigen was not temperature-sensitive in ts*A*-transformed or productively infected cells, Robb *et al.*[14] showed that cells productively and abortively infected by these mutants had decreased T-antigen expression at the conditional temperature, and Osborn and Weber[15] found that T-antigen decreased with time at the elevated temperature in ts*A*-transformed cells. Tegtmeyer *et al.*[13] found that, at the conditional temperature, the ts*A* mutants had an increased turnover of

Table 1* Induction of cellular and viral DNA synthesis in TC7 cells infected with wild type and *in*(ts)–1501 virus

Incubation temperature (°C)	Mock-infected		WT SV40-infected		*1501*-infected	
	Precipitate (c.p.m. per 10^5 cells)	Supernatant (c.p.m. per 10^5 cells)	Precipitate (c.p.m. per 10^5 cells)	Supernatant (c.p.m. per 10^5 cells)	Precipitate (c.p.m. per 10^5 cells)	Supernatant (c.p.m. per 10^5 cells)
37	1,101	27	34,892	9,450	38,923	12,850
41	1,572	101	26,750	13,867	37,872	10,667

* Cells, growing in 60-mm Petri dishes, were maintained in Eagle's minimal essential medium supplemented with 2 mM glutamine and 2% heat-inactivated (56 °C, 30 min) foetal bovine serum. Cell cultures were incubated in dark, water-saturated, 5% CO_2, temperature-regulated incubators. Virus was adsorbed for 1 h at 37 °C, followed by the addition of medium and then incubation at either 37° or 41 °C. The cells were labelled 20 h after infection with ^{3}H-dT (0.5 μCi ml^{-1} specific activity 6.7 Ci mmol^{-1}, New England Nuclear) for 90 min. DNA was selectively extracted by the method of Hirt[26]. The Hirt precipitate consisted of cellular DNA and the supernatant consisted of viral DNA. All determinations are average counts from triplicate Petri dishes.

T-antigen and an altered intracellular distribution in both productively infected and transformed cells at the elevated temperature. Tenen *et al.* [16] and Alwine *et al.*[17] found that T-antigen induced by ts*A* mutants is more thermolabile than that induced by WT virus.

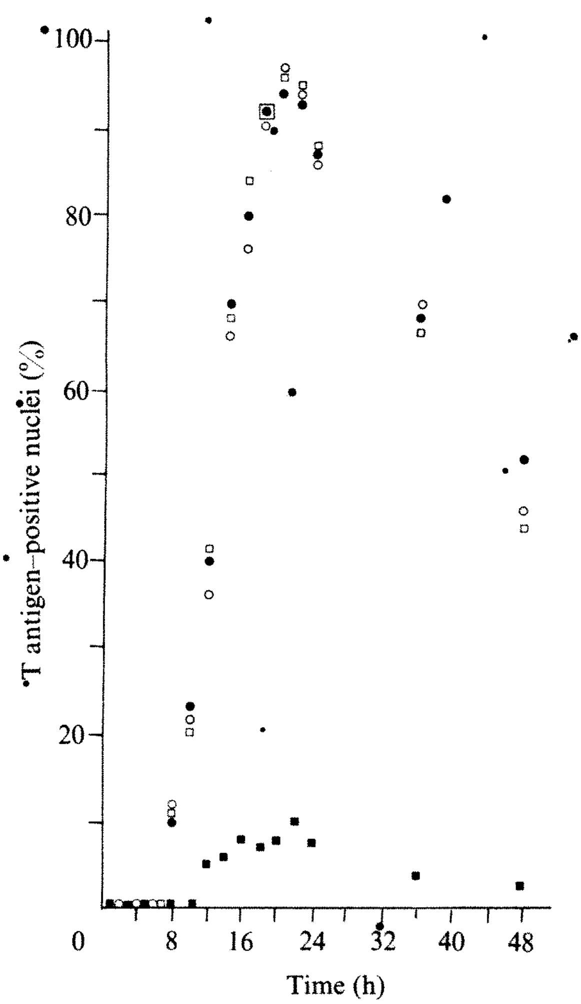

Fig. 1 Monolayers of African green monkey kidney cells (TC7) growing on coverslips in 60 × 15-mm plastic Petri dishes were infected with a multiplicity of infection of 10 by either wild type or *in*(ts)-1501 virus. After adsorption of the virus for 1 h, medium was added to the plates. Those cells infected with WT virus were incubated at 37 °C (○) or 41 °C (●). Plates with *1501*-infected cells were also incubated at 37 °C (□) or 41 °C (■). Each point represents the percentage of T-antigen-positive nuclei determined in 2,000 cells. Cells, fixed on coverslips, were stained for 30 min with hamster T-antiserum (Flow), diluted 1 : 10 in phosphate-buffered saline (PBS), and rinsed for 30 min in PBS. They were then incubated for 30 min at 37 °C after addition of 5 drops of fluorescein-conjugated rabbit anti-hamster IgG, diluted 1 : 10 in PBS, were added to each coverslip. The coverslips were then washed in PBS, mounted on glass slides with 1 drop of PBS-glycerine 1 : 10, *p*H 7.4, and examined with a Leitz fluorescence microscope. Each point represents the average of two experiments.

We studied T-antigen formation in WT SV40 and *1501* productively infected TC7 cells using a modification of the indirect immunofluorescence technique described by Pope and Rowe[19]. After virion adsorption at 37 °C for 60 min, T-antigen development was monitored in WT SV40-infected and *1501*-infected cells incubated at 37 °C and 41 °C (Fig. 1). More than 90% of the WT SV40-infected cells at both temperatures and *1501*-infected cells at 37 °C showed positive T-antigen fluorescence, but less than 10% mutant-infected cells were T-positive at 41 °C.

We also studied the effects of elevated temperature on T-antigen expression in WT SV40-transformed and *1501*-transformed hamster kidney cells. The histories and characteristics of T8-AP1, a hamster kidney cell line transformed by WT SV40, and HK10-c2 (*in*(ts)-1501), a twice-cloned, heat-inducible hamster kidney cell line transformed by 1501, were described previously[3]. Because Robb[20] showed that WT SV40-transformed and ts*D*-transformed 3T3 cells have reduced T-antigen expression at nongrowing saturation densities, we monitored T-antigen in subconfluent (60–70%) cultures. Unlike WT SV40-transformed cells, the *1501*-transformed cells had decreased T-antigen expression at the elevated temperature (Fig. 2). Increasing activation of infectious virus was observed in parallel cultures of *1501*-transformed cells at 41 °C but not at 37 °C nor in WT SV40-transformed cells at either temperature.

Table 2 Determination of T-antigen in cell extracts by antibody absorption

Incubation mixture	Titre
Buffer	1 : 8
WT SV40-transformed cell (37 °C)	0
WT SV40-transformed cell (41 °C)	0
1501-transformed cell (37 °C)	0
1501-transformed cell (41 °C)	1 : 8

Subconfluent cultures were grown at 37 °C and 41 °C for 48 h. T-antigen was extracted by the method of Ahmad-Zadeh *et al.*[27]. Cells were suspended at concentrations of 10^7–10^8 in 5 ml of extraction buffer (2.5 mM Tris–HCl, *p*H 7.4, 0.5M LiCl, 1 mM EDTA), sonicated three times for 15 s, and immediately centrifuged for 30 min at 30,000*g* and 4 °C. The supernatant was removed and used as the antigen. Extracts were adjusted to the same protein concentration (20 mg ml^{-1}). Antigen was assayed by the ability of the cell extract to absorb antibody to T-antigen. A modification of the method of Alspaugh *et al.*[28] was used. Equal volumes of anti-T serum and the cell extracts were incubated at 37 °C for 60 min, then at 4 °C for 16 h. Incubation mixtures were then serially diluted and assayed for unbound T-antibody by indirect immunofluorescence on WT SV40-transformed cells. The titre represents the highest dilution of the incubation mixture that still gave the chracteristic nuclear speckling of T-antigen-positive cells.

The presence of T-antigen was also assayed by using antigen extracts from each cell line grown at 37 or 41 °C to absorb the the T-antibody from SV40 T-antiserum before staining for immunofluorescence (Table 2). If T-antigen was present in the extract, it would bind to the T-antibody and prevent immunofluorescent staining of WT SV40-transformed cells. The immunofluorescence titre was decreased eightfold or greater by the extracts from *1501*-transformed cells grown at 37 °C and

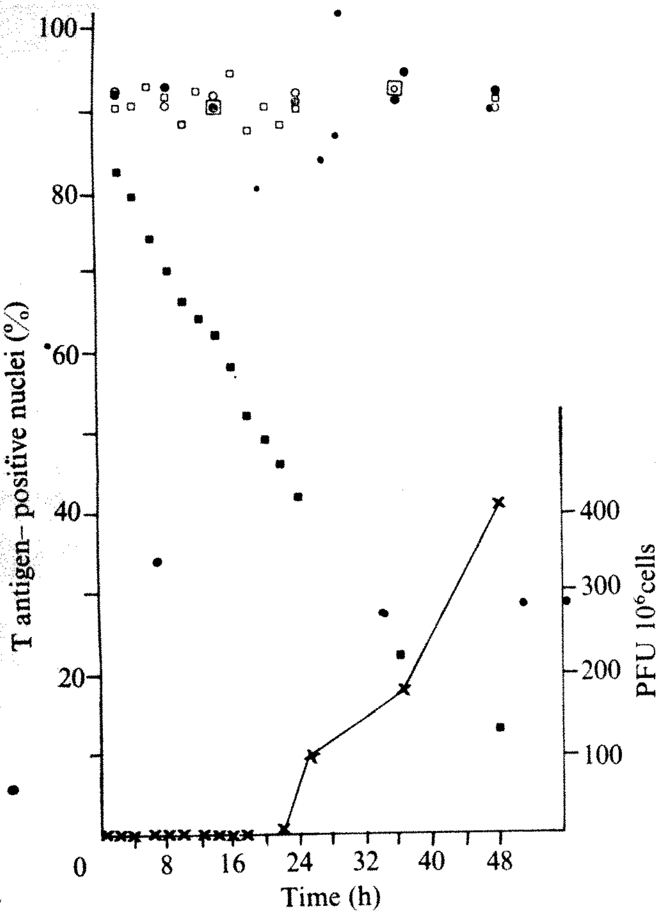

Fig. 2 All cell lines were maintained at 37 °C for 24 h after passage. At time zero, WT-transformed cells were incubated at 37 °C (○) and 41 °C (●). Parallel cultures of *in*(ts)-1501-transformed cells were incubated at 37 °C (□) and 41 °C (■). Infectious virus (×) activated at 41 °C in *1501*-transformed cells was assayed as plaque-forming units (PFU). The percentage of T-positive cells was determined as described in the legend of Fig. 1. The cell viability, measured by Trypan blue exclusion, remained more than 92% throughout the experiment. *in*(ts)-1501-transformed cells exhibited a characteristic decline in viability after 96 h at 41 °C.

from T8-AP1 grown at either temperature; however, the extract from *1501*-transformed cells grown at 41 °C did not absorb the antibodies in the T-antiserum, indicating that T-antigen is not present in these conditions.

Thermal lability of the *1501* T-antigen extract was assayed by heating aliquots of the extract for various times at 50 °C. Using the antibody-absorption technique described in Table 2, we found that T-antigen was completely inactivated after 30 min at 50 °C.

We tested for the reversability of the apparent loss of T-antigen in HK10-c2(*in*(ts)-1501) cells at the nonconditional temperature. Subconfluent cultures of cells, maintained at 41 °C for 24 h, were shifted to 37 °C. The percentage of T-antigen-positive nuclei gradually increased from 38% after 24 h at 41 °C to 90–95% after incubation at 37 °C for 3 d. Cell viability, measured by trypan blue exclusion, remained greater than 90% throughout the experiment.

These results suggest that the *in* gene affects the heat lability of T-antigen and that certain antigenic determinants of T-antigen may be required for the maintenance of SV40 DNA integration. Furthermore, induction of host-cell DNA synthesis and T-antigen are separate early viral functions. We postulate that one function of the SV40 T-antigen may be to interact with specific recognition sites on either viral or cellular DNA, selectively regulating viral DNA integration and/or late viral DNA transcription. Consistent with this hypothesis are the findings of Carrol *et al.*[21], who demonstrated that SV40 T-antigen binds strongly to double-stranded DNA with two different salt-concentration-dependent affinities, and of Reed *et al.*[22], who showed that T-antigen can bind selectively to SV40 DNA at or near the origin of SV40 DNA replication.

We must reconcile our observations with findings that the T-antigen is the *A*-gene protein[13,16,17,23]. The thermal lability of T-antigen from *1501*-transformed cells indicates a relationship between the *1501* and ts*A* mutants. The most likely explanation is that the *in* and *A* cistrons may code for a multifunctional protein, such as that found in the his*B* gene in *Salmonella typhimurium*[24] or the aspartokinase I-homoserine dehydrogenase I gene of *E. coli*[25], in which two activities can be altered independently. Other explanations are possible, however. A test of the hypothesis of T-antigen pleiotropy will necessitate the isolation and characterisation of the mutant T-antigen and study of its interaction with DNA.

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Contiguity to male foetuses affects morphology and behaviour of female mice

DURING studies on the role of gonadal hormones in aggressive behaviour of mice, we observed large variations in both the morphology and behaviour of females. At birth, variation was evident with respect to the principal external sex-differentiating marker—distance between the anus and the genitals, with some females being difficult to distinguish from males. Moreover, a significant proportion of females, upon reaching adulthood, were more responsive to androgen than others, exhibiting male-like aggressive behaviour more quickly following its administration. These observations led to the speculation that a certain proportion of females had been masculinised naturally during prenatal development. We present here data which show that such masculinisation does occur and that it is mediated by proximity to male foetuses.

That prenatal masculinisation of the female can occur naturally in the rodent was suggested by the data of Clemens[1]. Based upon a relatively small sample, especially with respect to that used to assess behaviour, it was reported that female rats, contiguous to male foetuses during gestation, had larger ano-genital distances at delivery and displayed more male sex behaviour in response to androgen treatment given in adulthood than did females that had not been in such close proximity to males. It was suggested that masculinisation of the female was mediated by exposure to androgen from the foetal testis. We now present data which show conclusively that, in mice, the position of the female in the uterus relative to that of males determines the extent to which the female is both morphologically and behaviourally masculinised.

Thirty-eight 60–70 d-old nulliparous Rockland–Swiss albino mice, housed separately in 11 × 7 × 5-inch translucent cages, were provided with food and water in excess and kept on a 12 h light/12 h dark cycle with lights on at 0600 h. An adult male was introduced into each female's cage and removed on the discovery of a copulatory plug. Pregnancy was interrupted at 2000 h on day 18 of gestation (delivery normally occurs between 0100 and 0500 h on day 19 of gestation, taking the day of coitus as day 0 of pregnancy) and the foetuses were delivered surgically. The adult was killed by cervical dislocation, the uterine horns were exposed, and the foetuses expelled with their position in the uterus relative to other foetuses noted. They were cleaned with saline and ano-genital distances measured. The females then were gonadectomised under ether anaesthesia and marked by injecting India ink into the footpads. The sequence of marks identified the position of a foetus with respect to the sex of the foetuses on either side. Males from 10 of the pregnant animals also were gonadectomised and marked. The animals then were fostered in same-sex groups of four or five to mothers that had delivered within the preceding 24 h and whose young had been removed.

The animals were weaned on day 21 of life and kept in their foster-littermate groups until day 60, when they were implanted subcutaneously with a 10-mm Silastic capsule containing 5 mg testosterone (T) and housed singly. Testing for aggression, begun 2 d later, was conducted on alternate days for a maximum of 50 d (25 tests) or until a fight occurred. Testing consisted of placing an adult olfactory bulbectomised male into the cage of each female and leaving it there for 10 min. Olfactory bulbectomised males, although eliciting aggression comparable to that of intact males, neither initiate fights nor respond to attack by retaliating. Thus, they provide a relatively constant stimulus for tests of aggression[2]. A fight was scored when an animal chased and bit the stimulus male. The number of days of T exposure required to induce aggression was recorded. Although the administration of T to non-neonatally androgen-exposed adult mice will produce aggression, animals exposed to T during early development will fight sooner following the start of T exposure in adulthood[3].

Fifty-two females survived to adulthood: 15 had been situated between two male foetuses (group 2M), 28 next to one male foetus (group 1M), and nine contiguous with no male foetus (group 0M). Nineteen males also survived to adulthood. An analysis of variance of the ano-genital distances of the females recorded at delivery yielded a significant uterine position effect ($P > 0.001$) (see Fig. 1). Individual comparisons using Duncan's test[4] with α at 0.05 showed that animals of group 2M had significantly larger ano-genital distances than did those of group 1M and 0M. The latter two groups did not differ from one another. As it was possible that the absolute number of males in the uterus rather than contiguity to males mediated the effect upon ano-genital distance, an analysis of covariance was performed with the number of males per uterus serving as the covariate. This analysis revealed that ano-genital distance did not vary as a function of the absolute number of males present in a uterus. The mean ano-genital distance for each of the 3 groups of females was then compared to the mean for the males using Dunnett's test[4] to compare means to a standard with α at 0.05. Males had a significantly larger mean ano-genital distance than did any of the groups of females.

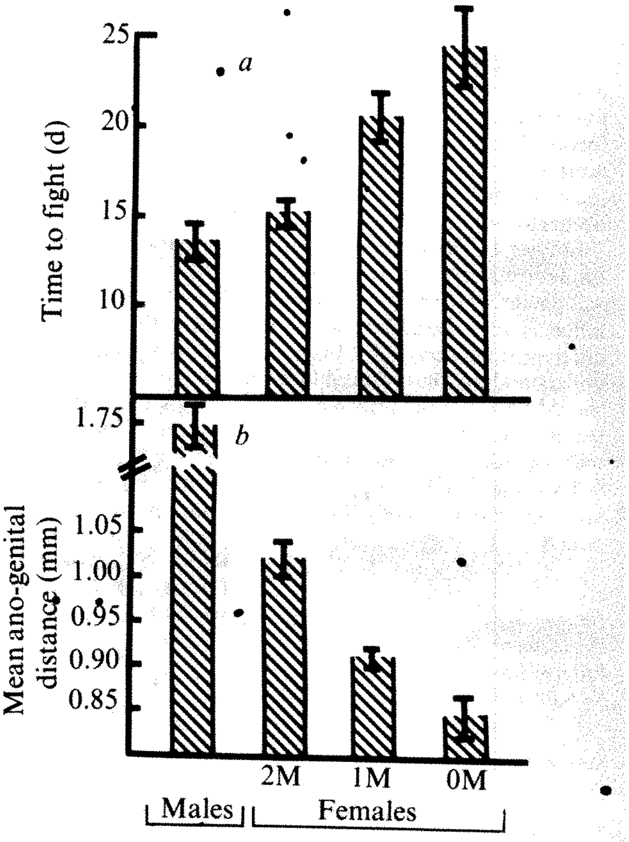

Fig. 1 *a*, Number of days to fight following the start of testosterone exposure of male and female mice, and *b*, ano-genital distance of delivery. Females were located in the uterus either between two male foetuses (2M), next to one male (1M), or contiguous to no male foetus (0M). All animals were delivered approximately 3.5 h prematurely and gonadectomised at that time. Data are means ± s.e.m.

As ano-genital distance varied as a function of contiguity to male foetuses, it was predicted that the females of the most morphologically masculinised group (2M), with the largest ano-genital distance, would require the shortest period of exposure to T in order to establish aggression. Conversely, females of the least masculinised group (0M) would require the longest exposure period. These predictions were supported. Females of Group 2M did require a significantly shorter period of exposure to T to exhibit aggression than did animals of group 0M. Females of group 1M were intermediate, not differing significantly from the animals of group 2M and 0M. The males did not differ significantly from the females of group 2M and 1M but did require a shorter exposure period to T than animals of group 0M. Finally, collapsing across the three groups of females, a significant negative correlation ($r = -0.30$, $P < 0.05$) was obtained between ano-genital distance at delivery and the number of days of T exposure required to induce aggression.

The variability normally exhibited by female mice in morphology and behaviour can thus be accounted for, in part, by the position of the female in the uterus relative to that of males. Females situated between two male foetuses were masculinised as indicated by larger ano-genital distance and increased responsiveness to the aggression-promoting property of T. Females contiguous with one male were masculinised with respect to behavioural responsivity to T but not morphologically. Female rat foetuses contiguous to a male foetus are heavier[5], so the large ano-genital distance of female mice situated between two males might have been due to the fact that these females were heavier than the others. This is not the case, however, as there were no differences in body weight between the three groups of females. It also is possible that the effect is specific only to the Rockland–Swiss strain. Although data on the effect of uterine position on responsiveness to T are not available, we have found that uterine position does significantly influence ano–genital distance in the CF-1 strain.

The foetal mouse testis begins to secrete androgen on day 12.5 of gestation[6], and tritiated T injected into the amniotic fluid of a rat foetus passes by diffusion into the amniotic fluid of a contiguous foetus[7]. This indicates, therefore, that the effect upon female morphology and behaviour of proximate male foetus may be mediated by exposure to androgen and that its transmittal occurs locally at the level of adjacent amniotic membranes.

Studies of the difference between disparate groups provide fundamental information concerning factors which affect development, as in the use of males and females to demonstrate the influence of gonadal hormones. Identifying the determinants of differences between individuals, however, is a prime requisite for understanding the variability within a group and, therefore, should be one of the principal concerns of psychology. Our study seems to identify a contributory factor underlying the development of individual differences.

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Mutagenicity and toxicity of visible fluorescent light to cultured mammalian cells

MANY cultured mammalian cells are characterised by karyotypic instability, high rates of spontaneous transformation and high frequencies of background mutation. The toxicity of visible light for mammalian cells[1–4] is usually attributed to photochemical reactions between light and the culture medium, and riboflavin, tryptophan and tyrosine are often thought to mediate this toxicity[1–3]. Light has been shown to produce strand breaks in DNA[5,6]. Because many tissue culture operations are conducted under fluorescent light, we have investigated whether the toxicity and DNA strand-breaking activity of light are accompanied by mutagenicity. We report here that this is the case.

The toxicity of fluorescent light for V-79 Chinese hamster lung (CHL) cells incubated during irradiation in either complete α-MEM (CM) or balanced saline solution (BSS) is shown in Fig. 1. Both survival curves are slightly shouldered, implying a saturable repair process. Evidently light is toxic to these cells whether they are irradiated in CM or BSS, although toxicity after 2 h of irradiation is greater for cells in CM. This result is in contrast to those of Stoien and Wang[2], who found no killing of human D98/AH2 cells irradiated with fluorescent and near-ultraviolet light in phosphate-buffered saline.

We tested whether pre-irradiation of CM, toxic for some cells[2,7], would kill V-79 cells subsequently incubated in it. Table 1 shows that medium that has been pre-irradiated for 3 h in the same conditions as the cells does not reduce the colony-forming ability of V-79 cells. The final colony diameter after 1 week of growth, however, was decreased and the cellular morphology was altered by pre-irradiated CM (Table 1).

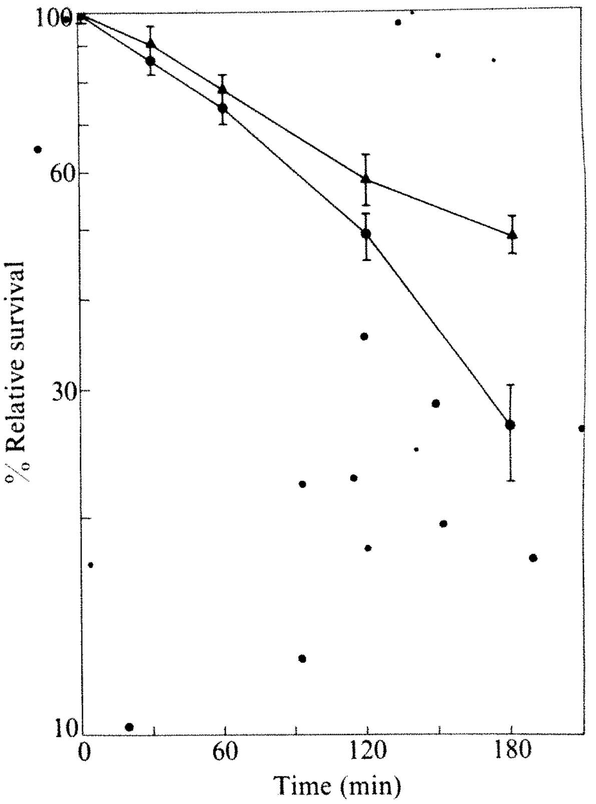

Fig.1 Toxicity of fluorescent light to V-79 CHL cells incubated in medium or saline. Three hundred exponentially growing V-79 cells were inoculated into 10 ml of CM (α-MEM[8], minus purines and pyrimidines, containing 5% FCS (Flow Laboratories) and 25 mM HEPES in 100-mm Falcon plastic Petri dishes). The cells were allowed to attach to the dishes at 37 °C in a 7.5% CO$_2$/air incubator. Four hours after inoculation, some plates were rinsed three times with BSS (without phenol red) containing 25 mM HEPES and incubated at 4 °C in the same solution. Plates containing CM or BSS were placed in a room at 4 °C and irradiated with fluorescent light for the times indicated. All plates were kept in the cold room for 3.5 h. Irradiation was by four Sylvania Cool White F15T8-CW bulbs held in two desk lamps. The bulbs were 8 cm above the surface of the cells and the intensity of illumination at that distance was 1,700 foot candles as measured with a Weston Foot Candle Meter Model 614. Irradiation was from above through both the plastic dish top and the overlying medium. The temperature in the dishes never exceeded 10 °C. After 1 week of incubation, the colonies were rinsed with BSS, fixed in methanol for 10 min, stained with Giemsa and counted. A colony was defined as more than 64 cells in close proximity. The plating efficiency of the unirradiated chilled control cells in CM was $86\pm3\%$ (six plates) and in BSS it was $82\pm7\%$ (six plates). The number of colonies surviving after light treatment is normalised to the survival of the controls. The mean relative survival $\pm$ % s.d. is plotted. When the standard deviations overlap, the upper and lower limit bars are for the upper and lower points, respectively: ●, cells irradiated in CM; ▲, cells irradiated in BSS.

These data suggest that the basis of the toxicity of fluorescent light to V-79 cells is primarily a direct action of light on the cells themselves, because toxicity occurs in BSS alone (Fig. 1) and because preirradiated medium does not reduce colony-forming ability (Table 1). Nevertheless, complete medium can potentiate the action of light as shown by the slightly greater killing of cells in medium compared with saline (Fig. 1) and the reduced colony diameter and altered cell morphology caused by pre-irradiated CM (Table 1).

The effect of fluorescent light on the frequency of mutants in V-79 cells is shown in Fig. 2. Resistance to 6-thioguanine was used as a marker for inactive hypoxanthine-guanine phosphoribosyl transferase and as an assay for mutant colonies. The method is described in the legend to Fig. 2. The number of 6-thioguanine-resistant, presumably mutant, colonies increased linearly as the time of exposure to cool white fluorescent light

Table 1 Toxicity of pre-irradiated medium to V-79 Cells

Time of pre-irradiation (h)	% Colony formation relative to controls	Mean colony diameter (mm)	Cellular morphology
0	100±7	0.96±0.38	Normal
1.3	101±5	0.66±0.29	Abnormal
3.0	99±3	0.57±0.36	Abnormal

Three hundred V-79 cells were plated in 100-mM Petri plates as described in the legend to Fig. 1, except that no HEPES was included in the medium. Fifty ml of CM in a closed Corning 150-cm² flask in a room at 4 °C were irradiated with the fluorescent lights described in Fig. 1. To test the effect of irradiating CM on cloning efficiency, the original seeding medium was replaced after 4 h with the same volume of irradiated medium. After 7 d of incubation, colonies were fixed with methanol and stained with Giemsa. The plating efficiency of the controls was 78±3%. The values shown represent the mean survival relative to the controls ± s.d. for three plates. The mean colony diameter and s.d. were determined by measuring 50 colonies on three plates. The differences between the light-treated and untreated means are significant by a *t* test with a *P* value of 0.01. Cellular morphology was examined along the edges of clones with a phase contrast microscope. Morphology was considered abnormal if the cytoplasm was elongated and vacuolated in many clones.

increased (Fig. 2). After 3 h of irradiation in α-MEM the frequency of mutants was 166 times greater than in unirradiated controls. The single point above the line in Fig. 2 shows that after 3 h the frequency of mutants was greater for cells irradiated in BSS than for cells irradiated in CM. This is confirmed by the data in Table 2, which show that fluorescent light was 1.9 times more mutagenic for cells irradiated for 1 h in BSS than for cells irradiated for 1 h in CM.

If 50 ml of CM was used as a filter in a 150-cm² flask placed over the cells in another 150-cm² flask containing 50 ml of BSS, the frequency of mutants was not significantly changed from cells irradiated in CM without a filter (Table 2). This experiment implies that irradiation in CM is less mutagenic than irradiation in BSS because CM is partially filtering mutagenic portions of the fluorescent lamp emission.

Assuming that a linear dose–response relationship exists for mutagenicity with shorter exposures to light (Fig. 2), it can be calculated from the data presented here that a 1-h exposure to the incident fluorescent light in our tissue culture room (60 foot candles) will double the frequency of mutants in V-79 cells. In most laboratories this amount of exposure or more would occur intermittently on a daily basis. For example, we have found that after killing pre-existing 6-thioguanine-resistant mutants with HAT medium, the frequency of spontaneous mutants will increase progressively during a period of weeks (our unpublished results). This observation might be explained by the mutagenic action of the fluorescent light in our tissue culture hood and culture room that causes the frequency of mutants to increase with each exposure. Therefore it is possible that other genetic instabilities seen in cultured cells, such as malignant and karyotypic alterations, might be accounted for in part by the action of fluorescent light on the cellular DNA.

Table 2 Mutagenicity of fluorescent light for cells irradiated in CM or BSS

Medium	Filter	Time of irradiation (h)	Frequency of mutants* $\times 10^{-6}$
CM	—	0	1.42± 0.21
CM	—	1	38.6± 6.4
BSS	—	1	71.9±12.4
BSS	50 ml CM	1	34.1± 5.1

The frequency of mutants was determined as described for Fig. 2.
*Mean value±s.d. of three determinations.

From the data presented here, we conclude that exposure to fluorescent light should be limited during virtually any study with cultured cells. It is important that all exposure to light in our work was through the plastic tops of the culture vessels. Plastic absorbs more than 99% of any ultraviolet wavelengths less than 295 nm, so that the active wavelengths are presumably

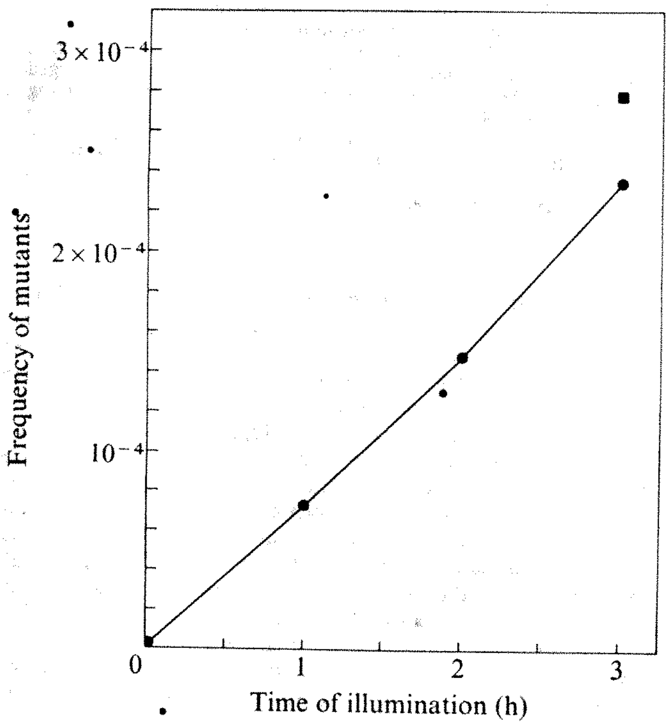

Fig. 2 Mutagenicity of fluorescent light to V-79 CHL cells incubated in CM or BSS. The frequency of mutants in V-79 cells after various treatments was measured by a modification of a method described by Myhr and DiPaolo[9]. Briefly, cells were cultured in α-MEM with 5% FCS at 2.5×10^5 cells per 150-cm² Corning plastic flask or per Falcon 15-cm Petri dish. Stock cells were subcultured every Monday and Thursday. On Thursday of each week, 2.5×10^5 stock cells were placed in HAT medium (CM containing 10^{-5}M hypoxanthine, 3.2×10^{-6}M aminopterin and 5×10^{-6}M thymidine,) and subcultured into HAT medium on the next Monday. On the following Thursday, having been in HAT medium for 1 week, cells were trypsinised and incubated in HT medium (CM containing 10^{-5}M hypoxanthine and 5×10^{-6}M thymidine). On Friday the medium was changed to CM; on Monday HAT-HT-CM cells were inoculated (10^6 cells each) into 150-cm² flasks in 50 ml of CM. On Wednesday there were usually 3×10^7 cells in log phase in each flask. One flask was trypsinised, and the cells were inoculated into 100-mm Petri dishes to determine the toxicity of the light treatment. The other flasks were irradiated in a room at 4 °C with fluorescent lights as described for Fig. 1. All flasks including controls were pre-incubated at 4 °C for 15 min before irradiation and remained in the cold room for 3.5 h until irradiation was complete. After irradiation the medium was removed and replaced with fresh CM at 37 °C. Two days later cells from the treated and control flasks were subcultured into 150-cm² flasks at 2×10^6 cells per flask to allow further time for mutations to be expressed. The flasks were incubated for at least another 3 d, until cultures were about to become confluent (at about 5–10× 10^7 cells) and had undergone at least four population doublings. If the cultures were not approaching confluency within 5 d of treatment, they were incubated until confluency was almost achieved. The fraction of mutant cells in the population at this time was assayed by seeding a total of 2.7×10^6 treated and recovered cells among nine 100-mm Petri dishes (3×10^5 cells per dish), in 15 ml of CM containing 6-thioguanine (11 μg ml⁻¹). At the same time, the plating efficiency of these cells was determined by seeding 300 cells each in six 100-mm Petri dishes in CM. Plates were incubated for 1 week before fixing and staining. A colony was defined as containing more than 64 cells. The data for the cultures incubated in CM during exposure to light were analysed statistically using linear regression by the method of least squares. The coefficient of determination for these data is 0.9975; this value is so close to 1.0 that these data can be said to fit a linear equation of the form $y = a_1x + a_0$, where a_1 and a_0 are constants. ■, Culture incubated in BSS during 3 h exposure to light; ●, cultures incubated in CM during 1, 2 or 3 h exposures to light.

longer than 295 nm. Exposure to excessive fluorescent light might produce artefacts in studies of *in vitro* toxicity, mutation, transformation, chromosome variation, ageing, effects of radiation, drug interactions and biochemistry. Whether or not our findings with V-79 Chinese hamster cells will extend to human and other mammalian cells remains to be determined. If human cells are also caused to mutate by fluorescent light, then the advisability of limiting human exposure to fluorescent light should be considered.

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Skin fibroblasts from a D-deletion type retinoblastoma patient are abnormally X-ray sensitive

RETINOBLASTOMA is a rare malignant eye tumour which appears spontaneously or occurs in genetically predisposed persons[1-3]. The latter group is composed of patients who inherit the tumour with a dominant mode of transmission (80–90% penetrance) (referred to as the familial type) or those who bear a deletion in the long arm of chromosome 13 (13q-) (referred to as the D-deletion type). When this deletion is present, it is observed in many somatic cells and is often associated with structural defects such as microcephaly, micropthalmia and several skeletal and genitourinary abnormalities[3,4]. Survivors of the genetic forms of retinoblastoma have an increased risk for the development of cancers at other sites, while patients who survive the sporadic type do not[2-6]. A single genetic locus is unlikely to predispose many somatic cells to tumour formation unless a fundamental molecular defect—for example, related to DNA repair—is present. To investigate this hypothesis, we have examined the *in vitro* X-ray sensitivity of skin fibroblast strains derived from three retinoblastoma patients—a pair of twins with the familial type accompanied by no gross chromosome abnormalities, and a patient with the D-deletion type. We also used two normal fibroblast strains and a strain from a patient with ataxia telangiectasia. We found that the strain derived from the D-deletion retinoblastoma patient was significantly more radiosensitive than the two normal strains, but not as sensitive as that from the ataxia patient.

Figure 1 shows the X-ray survival curves obtained with skin fibroblasts derived from both a normal individual and a patient with a D-deletion type retinoblastoma[3]. Also included for comparison is a survival curve for cells from a patient with ataxia telangiectasia; this autosomal recessive disease is associated with marked cellular radiosensitivity *in vitro*[7], presumably due to an X-ray repair defect[8]. As Fig. 1 shows, the retinoblastoma cells were significantly more sensitive to killing by X rays than were the normal fibroblasts, but not as sensitive as the ataxia cells.

Table 1 presents a comparison of X-ray survival curve parameters for the three retinoblastoma cell strains, two normal strains and the ataxia strain. The experiments were all performed within 10 weeks using the same lot of serum. Some variations were seen in survival parameters with different serum lots, but the sensitivities of these strains relative to each other remained constant. In nine experiments, including those in Table 1, the D_0 (inverse of the slope) for GM1142 ranged from 70 to 98 rad. The two familial retinoblastoma strains showed radiosensitivities intermediate between the D-deletion retinoblastoma strain and the normal strains, but it is not clear whether these retinoblastoma strains are significantly more radiosensitive than normal controls. As we have found before[9], the n values of the survival curves bore no relationship to radiosensitivity as measured by D_0. We have no explanation for the possible differences in n among the cell strains in Table 1. In preliminary experiments, all three retinoblastoma strains appeared normally sensitive to 254 nm ultraviolet irradiation.

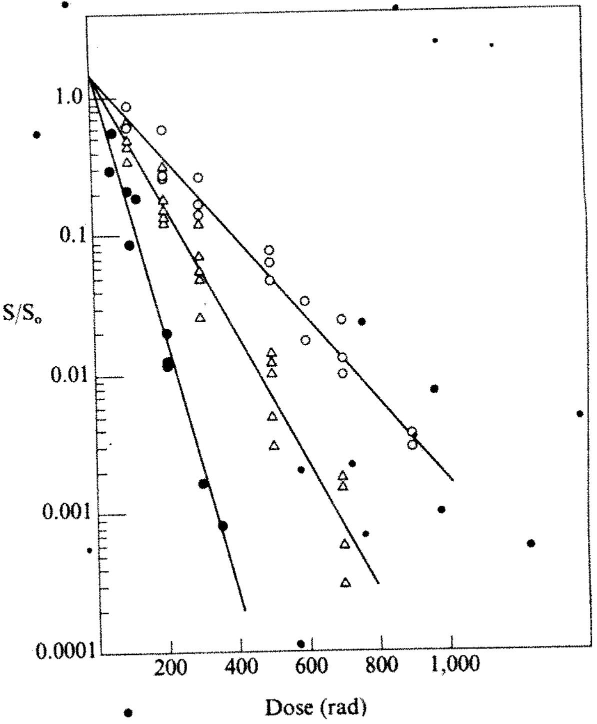

Fig. 1 X-ray survival curves for exponentially growing cultures. △, GM1142, skin fibroblasts from a female retinoblastoma patient with a deletion in the long arm of chromosome 13 (46, XX (13q-)); ●, CRL1343, skin fibroblasts from a patient with ataxia telangiectasia; ○, Li106, normal skin fibroblasts. Cultures were maintained at 37 °C in Eagle's minimal essential medium (Gibco F-15) supplemented with 15% foetal calf serum (Flow) D-glucose (900 mg l^{-1}), sodium pyruvate (0.66 mg l^{-1}) and Aureomycin (50µg ml^{-1}, Lederle) in a 5% CO_2 atmosphere. Cells from each strain were studied at similar passage levels in culture (ranging from 4th to 22nd mean population doubling). For experiments, exponentially growing cells were detached with 0.25% trypsin and seeded at appropriate numbers into 100-mm plastic Petri dishes. Dishes were X-irradiated 18 h later using a 220 kVp GE Maximar unit operated at 15 mA and yielding a dose rate of 80 rad min^{-1}. Dishes were returned to the incubator, the medium was changed after 7 d and the colonies were fixed and stained after 14 d. Cloning efficiencies ranged from 1 to 16%; radiosensitivity was not related to cloning efficiency (Table 1), and previous results have shown no relationship between cloning efficiency and survival curve parameters in several strains of human skin fibroblasts[9]. Points represent the average of triplicate plates. Curves represent a least squares linear regression analysis of the points shown. S/S₀, surviving fraction.

As more fibroblast strains from D-deletion retinoblastoma patients become available, it will be interesting to examine their responses to radiation in order to establish whether the radiosensitivity of the retinoblastoma strain described here relates to the fundamental disease process itself or to its

Table 1 X-ray sensitivities of human diploid fibroblast strains

Strain (source)	Clinical classification	Age/sex of donor	No. of experiments	Cloning efficiency	n*	D_o*
GM1142 (IMR)	Retinoblastoma D-deletion type	2 yr/F	6	0.7–6.5%	1.41±0.3	94±5
GM1131 (IMR)	Retinoblastoma familial type	9 mon/F	3	3.0–16.5%	0.87±0.09	132±4
GM1123 (IMR)	Retinoblastoma familial type (twin of GM1131)	9 mon/F	3	1.2–12.4%	1.03±0.1	121±4
CRL1343 (ATCC)	Ataxia telangiectasia	7 yr/F	3	0.7–1.8%	1.39±0.4	46±3
GM1381 (IMR)	Normal infant	2 mon/F	3	0.6–1.7%	0.99±0.1	148±9
Li106 (primary culture)	Normal adult	46 yr/M	6	1.0–6.3%	1.44±0.2	149±7

The data were obtained as described for Fig. 1.
IMR, Institute for Medical Research, Camden, New Jersey; ATCC, American Type Culture Collection, Rockville, Maryland; D_o, inverse of slope; n, extrapolation number to vertical axis.
*Mean ± 1 s.e. from least squares linear regression analysis of all data.

association with an abnormal karyotype involving a specific chromosomal region and perhaps a specific gene product. Patients with familial retinoblastoma may bear a much smaller deletion not detectable by available karyotypic and banding methods.

It is tempting to speculate that a specific X-ray repair function is governed by a portion of chromosome 13. Thus the predisposition to cancer in these patients might be related to a fundamental defect in a particular DNA repair system, analogous to the relationship between a deficiency in ultraviolet light repair and the predisposition to skin tumours in patients with xeroderma pigmentosum[10].

We conclude that skin fibroblasts derived from a patient with the D-deletion variant of retinoblastoma are abnormally radiosensitive. Perhaps future investigations will delineate a specific defect in molecular repair of DNA that will explain the predisposition of these patients to develop second primary tumours.

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Inhibition by sera and soluble antigens of T-cell-mediated cytotoxicity against leukaemia-associated antigens

INTEREST in the regulation of T-cell-mediated cytotoxicity by the major histocompatibility complex (*MHC*) genes stems from the discovery of (1) an H–2 restriction of T-cell-mediated cytotoxicity, that is sensitising cells, effector cells and target cells need to be syngeneic at the *H-2* locus, especially at the D and or K regions of the MHC[1–5] for effective killing and (2) inhibition of T-cell-mediated cytotoxic reactions against syngeneic tumours with alloantibodies to H–2 antigens specific for the tumour cells[6,7]. Different origins have been proposed for the tumour-associated antigens (TAA), and the most favourable model is a modified H–2 antigen or an adaptor–antigen complex[4–7]. This issue has important implications for tumour immunology, and so we have evaluated the validity of the inhibition of cytotoxicity by serum at the effector phase level; investigated afferent interference by serum of the cell-mediated cytotoxic response, and studied the inhibition of cell-mediated cytotoxic reactions with soluble antigens. We report here that there is a lack of H–2 restriction of T-cell-mediated cytotoxicity to TAA, and that TAA seems to differ from H–2 antigens. This implies that T-cell recognition is not entirely regulated by the *MHC* genes.

A 20-h ^{125}IUdR release assay (IRA) (ref. 8) was used to measure cell-mediated cytotoxic response to FBL-3 (ref. 9), a syngeneic Friend virus-induced lymphocytic leukaemia in C57BL/6 (B6) mice. The cytotoxic reactions were found to be primarily directed against the Friend Moloney-Rauscher (FMR) leukaemia-associated antigens[10–12]. Both the induction phase and the effector phase of these cytotoxic reactions were entirely T-cell-dependent. Pretreatment of the responding cells[10] or the effector cells[11,12] with anti-θ antibody plus complement eliminated cytotoxicity. Furthermore, removal of adherent cells and macrophages by passage of the effector cells through a rayon column or incubation in a Petri dish did not affect the level of cytotoxicity[11,12]. Thus the reactions were apparently not macrophage-mediated. When various antisera and normal sera were used to study their effects on these cytotoxic reactions (Table 1), we found that effector cells which were generated in B6 mice (H–2^b) by immunisation with syngeneic FBL-3 cells killed both the syngeneic target FBL-3 and an allogeneic target HFL/d-tc, a non-producer line of Friend virus-induced leukaemia in BALB/c (H–2^d) mice[13]. In other experiments, effective killing of FBL-3 and HFL/d-tc cells could also be achieved by lymphocytes obtained in BALB/c mice which were immunised with syngeneic HFL/d-tc cells[14]. These lymphocytes gave even higher levels of cytotoxicity against the allogeneic target FBL-3 than against the syngeneic target HFL/d-tc. In our experiment, an alloantiserum with the appropriate specificity, and the syngeneic serum produced against FBL-3 both inhibited cytotoxicity. In addition normal mouse serum (NMS) gave inhibition against FBL-3 targets.

If NMS or normal rabbit serum were used as a baseline for calculations of cytotoxicity, various other sera, for example, anti-FBS, which bound to the foetal bovine serum coated on the surface of target cells and rabbit anti-mouse cell serum, also inhibited the reactions against both FBL-3 and HFL/d-tc. Anti-Ia, and rabbit anti-mouse Ig, inhibited the reaction against FBL-3. None of the last four sera in Table 1 should contain antibody against H–2 antigens. Thus, it seems that any antibodies that bind to the cell surface can interfere with the cytotoxic reactions, perhaps by steric hindrance. This conclusion was also suggested by the following results. (1) Pretreatment of either the target cells or effector cells

Table 1 Inhibition of cell-mediated cytotoxic reactions with sera

Serum	FBL-3 Net % lysis	FBL-3 Net % lysis(s)	FBL-3 P	HFL/d-tc Net % lysis	HFL/d-tc Net % lysis(s)	HFL/d-tc P
None	49			18		
NMS	32			15		
Anti-H-2^b	4	−28	< 0.01	17	2	
Anti-H-2^d	47	15		10	−5	< 0.01
Anti-FBL-3	19	−13	< 0.01	10	−5	< 0.01
Anti-FBS	14	−18	< 0.01	7	−7	< 0.01
Anti-Ia	27	−5	< 0.05	ND		
NRS	46			18		
RAM	−5	−51	< 0.01	8	−10	< 0.01
RAG	10	−36	< 0.01	23	5	

^{125}IUdR release assay was performed in Microtest II plates. E/T (ratio of effector : target cells) = 500/1, incubation time was 20 h. Effector cells were the pooled lymph node and spleen cells obtained from B6 mice immunised by subcutaneous inoculation of 5 × 10^6 FBL-3 cells 10 d before assay. Various sera were added to the test wells at final dilutions of 1/5. NMS, normal mouse serum. Anti-H-2^b was from BALB/c immunised with B6 skin grafts and spleen cells. Anti-H-2^d was from B6 immunised with BALB/c skin graft and spleen cells. Anti-FBL-3, B6 immunised with FBL-3. Anti-FBS, B6 immunised with foetal bovine serum. Anti-Ia was from (B10.A ×)F; immunised with B6 spleen, absorbed with B6 tumour EL-4 cells (given by Dr David Sachs, NIH). NRS, normal rabbit serum. RAM was from rabbit immunised with mouse tumour (SV40 tumour mKSA in BALB/c). RAG, rabbit anti-mouse Ig. Net % lysis = (total % lysis obtained with immune lymphocytes) − (total % lysis obtained with normal lymphocytes). Net % lysis(s) = (net % lysis obtained with immune lymphocytes plus antiserum) − (net % lysis obtained with immune lymphocytes plus normal serum). Welch distribution t test was used to determine statistical significance. The results were compared to net percentage of lysis obtained with immune lymphocytes plus normal serum (NMS or NRS).

with antisera for up to 4 h followed by a single wash did not reduce cytotoxicity (data not shown); whereas significant antibody binding to the FBL-3 cells could still be demonstrated after five washes (Fig. 1). This indicates that excess antiserum is needed for inhibition. (2) The degree of inhibition by the serum was in approximate proportion to the amount of the antibody bound to the cell surface (Fig. 1). The isotopic antiglobulin technique (IAT)[15], which measures the amount of antibody bound to the cell surface indicated that the four mouse sera tested gave relative binding to FBL-3 cells in the order anti-H–2^b > anti-FBS > anti-FBL-3 > anti-Ia. When these sera were used in the IRA to assay for their ability to inhibit the cytotoxicity against FBL-3, the same order of effectiveness was found, that is anti-H–2^b was most inhibitory, and anti-Ia was least inhibitory. The anti-H–2^d serum which did not bind to FBL-3 cells also gave no inhibition of cytotoxicity. These results suggest that inhibition by serum need not depend on binding to specific structures, but only on binding to the cell surface, and they are consistent with inhibition being due to non-specific steric hindrance. The effective inhibition of the cytotoxic reaction obtained with rabbit anti-mouse Ig serum supports this possibility although it is not clear why this serum binds to the target cells. In addition, the inhibition of the killing of H–2^d target (HFL/d-tc) by anti-H–2^d seems to be non-specific, because the killer cells were generated by syngeneic immunisation with FBL-3 (H–2^b) and therefore presumably are not directed against any H–2^d-associated antigens.

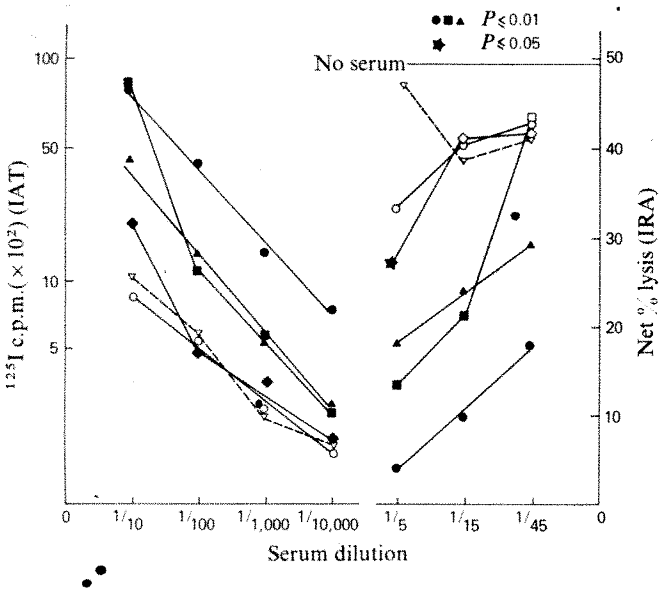

Fig. 1 Reaction of various sera to FBL-3 cells in the isotopic antiglobulin technique (IAT), and their effects on the cell-mediated cytotoxic reaction in the ^{125}IUdR release assay (IRA). In IAT, 0.1-ml samples of sera at various dilutions were incubated with 1 × 10^5 FBL-3 cells, then washed five times, followed by incubation with ^{125}I-labelled goat anti-mouse Ig. They were washed five times, and the radioactivity of the cell pellets were counted in a γ scintillation counter. The relative amounts of antibody bound to the cell surface were indicated as ^{125}I c.p.m. and were relative to that of normal mouse serum (NMS). In IRA, effector cells were obtained from B6 mice immunised with syngeneic tumour FBL-3 and tested against FBL-3 in Microtest II plates with E/T = 500/1 and incubation for 20 h. In this experiment, 0.1-ml samples of sera at various dilutions were added to the test wells to determine their effects on the inhibition of cytotoxicity. The statistics were evaluated by the Welch distribution t test. The results were compared to net percentage of lysis obtained with effector cells with addition of NMS. The mouse sera were the same as for Table 1. ∇, Anti H–2^d; ○, NMS; ★,⧫,◊ anti-Ia; ▲, anti-FBL-3; ■, anti-FBS; ●, anti-H–2^b.

We have shown that specific afferent interference of transplantation immunity to FBL-3 could be produced by pretreatment of the immunising cells with specific anti-tumour antiserum[16]. It follows that if TAA is truly modified H-2, then anti-H-2 antibody should produce similar interference. Therefore various sera were used to determine their effect on the induction of cell-mediated cytotoxic response to FBL-3 in the syngeneic B6 host (Table 2). Only pretreatment with syngeneic anti-FBL-3 serum could specifically reduce the cytotoxic response. Neither anti-H-2^b, nor anti-FBS had any effect, providing further evidence that TAA is not associated with H-2 antigen.

Table 2 Afferent interference of cell-mediated cytotoxic response with sera

Pretreatment*	Net % lysis obtained at E/T 200/1	Net % lysis obtained at E/T 40/1
Exp. 1		
None	26	3
NMS	28	5
Anti-FBL-3	14†	1
Anti-H-2^b	29	3
Anti-FBS	24	5
Exp. 2		
None	51	23
NMS	45	22
Anti-FBL-3	11†	2†
Anti-H-2^b	43	20
Anti-FBS	46	23

*1 × 10^5 of 10,000-R X-irradiated FBL-3 cells were incubated with 0.5 ml of undiluted serum at 37 °C for 60 min. Then 1 × 10^4 untreated or serum-treated cells were inoculated intraperitoneally into B6 mice. ^{125}IUdR assay was performed with peritoneal exudate cells 7 d after immunisation.

†$P \leq 0.01$: statistical significance was evaluated by the Welch distribution t test. The results were compared to net percentage of lysis obtained from mice immunised with untreated cells or normal serum-treated cells.

Finally, three different soluble antigens were tested for their effects on the cell-mediated cytotoxic reactions to FBL-3: papain-solubilised FBL-3 membrane antigen, papain-solubilised H–2^b antigen obtained from dissociated B6 spleen cells, and purified viral protein, gp70, prepared from Rauscher virus (given by Dr S. Oroszlan, Flow). The results are summarised in Table 3. Soluble FBL-3 antigen at concentrations as low as 7.5 μg ml^{-1} gave complete inhibition of the cytotoxicity; no inhibition could be obtained with H–2^b antigen even at 50 μg ml^{-1}. The purified gp70 gave 20–30% inhibition at 2.5–12.5 μg ml^{-1}. A mixture of gp70 and H–2^b antigen produced the same inhibition as gp70 alone. These data indicate that unaltered H–2^b antigenic specificities are not present on the receptor site recognised by these cytotoxic lymphocytes although modified H–2^b is not excluded as a target. The viral antigen gp70 is a cytotoxic target, but the fact that partially purified H–2^b does not enhance its inhibitory activity suggests that H–2^b-gp70 complexes are not usually targets, although such a complex might not be obtained easily in a cell-free system. The observation that the soluble FBL-3 preparation can totally inhibit cytotoxicity argues against the existence of H–2–tumour antigen complexes since this material is prepared by papain digestion and such non-covalent complexes would be unlikely to survive such harsh treatment.

Table 3 Inhibition of cell-mediated cytotoxic reactions with soluble antigens

Antigens	Concentration* (μg ml^{-1})	Net % lysis	% Reduction†
None		61	
FBL-3	25.0	−2	103
FBL-3	7.5	5	92
FBL-3	2.5	35	43
H–2^b‡	50.0	62	−1
H–2^b‡	10.0	62	−2
gp70	12.5	43	30
gp70	2.5	48	22
H–2^b+gp70	(50+12.5)	49	21
H–2^b+gp70	(10+ 2.5)	50	19

^{125}IUdR assay was performed as for Table 1.

*Final concentration of soluble antigen in test wells.

†% of reduction $= \dfrac{\text{(net \% lysis obtained with immune lymphocytes alone)} - \text{(net \% lysis obtained with immune lymphocytes plus soluble antigen)}}{\text{net \% lysis obtained with immune lymphocytes alone}}$

‡H–2^b was solubilised with papain and purified 200-fold from B6 spleen cells (M.J.R., unpublished data). When labelled, 40% of the preparation can be specifically precipitated with anti-H–2^b alloantisera.

We have demonstrated that efficient killing of allogeneic targets by T lymphocytes is generated by syngeneic immunisation (Table 1); that efferent inhibition of cytotoxicity by alloantisera can be non-specific (Fig. 1); that specific afferent inhibition of the cytotoxic response is only provided by syngeneic anti-tumour serum, not alloantisera (Table 2); that complete inhibition of cytotoxic reactions can only be obtained with soluble tumour antigen, not H–2 antigen (Table 3) and that cytotoxicity against syngeneic targets can be generated by allogeneic immunisation[14,17].

These data strongly suggest that there is no H–2 restriction of T-cell-mediated cytotoxicity to specific leukaemia-associated antigen(s) in this system. The findings are also contrary to the hypothesis of the TAA measured by our cytotoxicity assay being an (H–2) adaptor-(tumour) antigen complex. The discrepancy between these results and other reports of H–2 restriction could be due to differences in the assays. We have found that a 4–10 h ^{51}Cr release assay which was used by others[1–7], fails to detect the killing of allogeneic targets which may require longer incubation (20–40 h) (ref. 14). The explanation for this discrepancy is that *MHC* genes can play some role in the initial recognition phase of the cytotoxic reaction, thus enhancing short term killing in syngeneic target effector combination. The *MHC* genes are not essential, however, because recognition of specific, non-MHC antigens by allogeneic effector cells can produce effective cytotoxicity which becomes evident with longer incubation time.

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Active site of bovine factor XI (plasma thromboplastin antecedent)

FACTOR XI (plasma thromboplastin antecedent) is a plasma glycoprotein which participates in the early stages of blood coagulation[1]. A deficiency of this protein leads to a haemorrhagic disease in which the intrinsic pathway of blood coagulation is partially or completely blocked[2]. During normal coagulation, factor XI is converted to a serine protease (factor XI$_a$)[3], and this enzyme in turn activates factor IX (Christmas factor) by limited proteolysis[4]. Factor XI$_a$ is inhibited by serine protease inhibitors such as diisopropyl phosphorofluoridate, and this inhibitor is bound to a serine residue in the active site of the enzyme[3]. We recently purified bovine factor XI approximately 28,000-fold in 30% yield. The final preparation was

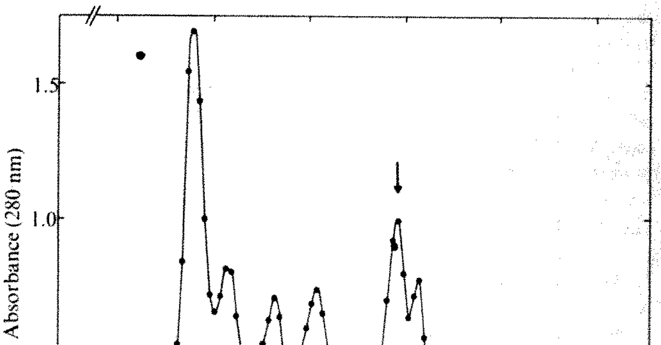

Fig. 1 Gel filtration pattern of the cyanogen bromide digest of *S*-pyridylethyl bovine factor XI. Factor XI, 40 mg, was pyridylethylated by the method of Friedman *et al.*[15] and the protein was separated from the salt and excess reagent by gel filtration on Sephadex G-50 (1.8 cm × 50 cm column) in the presence of 9% formic acid. Cyanogen bromide digestion was carried out by dissolving 40 mg of the salt-free *S*-pyridylethyl factor XI and 80 mg of cyanogen bromide in 2 ml of 70% formic acid, and the reaction was allowed to proceed for 24 h at 4 °C. After lyophilisation, the digest was fractionated by gel filtration on Sephadex G-75 (1.6 cm × 90 cm column) in the presence of 9% formic acid. The flow rate was 4.2 ml h^{-1} and fractions were collected in 1.4 ml per tube. The solid bar shows the fractions of peak 6 that were combined and lyophilised.

					180					185					190					195			
Factor XI	Val	Cys	Ala	Gly	Tyr	Arg	–	Glu	Gly	Gly	Lys	–	–	Asp	Ala	Cys	Lys	Gly	Asp	SER	Gly	Gly	Pro
Factor XII*	Leu	Cys	Ala	Gly	Phe	Leu	–	Glu	Gly	Gly	Thr	–	–	Asp	Ala	Cys	Gln	Gly	Asp	SER	Gly	Gly	Pro
Factor IX_a†	Phe	Cys	Ala	Gly	Tyr	His	–	Glu	Gly	Gly	Lys	–	–	Asp	Ser	Cys	Gln	Gly	Asp	SER	Gly	Gly	Pro
Factor X_a‡	Phe	Cys	Ala	Gly	Tyr	Asp	–	Thr	Gln	Pro	Glu	–	–	Asp	Ala	Cys	Gln	Gly	Asp	SER	Gly	Gly	Pro
Thrombin§	Phe	Cys	Ala	Gly	Tyr	Lys	Pro	Gly	Glu	Gly	Lys	Arg	Gly	Asp	Ala	Cys	Glu	Gly	Asp	SER	Gly	Gly	Pro

Fig. 2 Active site sequence of bovine factor XI and several other blood coagulation proteins. Amino-terminal sequence analysis was performed on 1.2-mg samples of peak 6, Fig. 1, with a Beckman sequencer Model 890A. The operation of this instrument and the methods used are adaptations[16] of Edman and Begg's technique[17]. Phenylthiohydantoin amino acids were identified by gas chromatography after silylation or directly by high-pressure liquid chromatography[18]. Amino acid residues in factor XI that are identical with other plasma serine proteases are shown in blocks. —, spaces inserted to bring the five proteins into alignment for better homology. The numbering system is that of chymotrypsin where the active site serine is residue 195. * Ref. 19; † ref. 20; ‡ ref. 21; § ref. 22.

homogeneous as measured by sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoelectrophoresis[5]. It has a molecular weight of 124,000 as determined by sedimentation equilibrium and is composed of two similar or identical polypeptide chains held together by a disulphide bond(s). We present here data showing that bovine factor XI contains an amino acid sequence which is very similar to the active site region of many other serine proteases involved in the coagulation process.

S-pyridylethyl factor XI was digested with cyanogen bromide and the polypeptide fragments were fractionated by gel filtration on Sephadex G-75 (Fig. 1). Twelve ultraviolet-absorbing peaks were obtained. Peak 6 (shown by the arrow) migrated as a single band on sodium dodecyl sulphate polyacrylamide gel electrophoresis and had a molecular weight of approximately 5,500. The amino-terminal sequence of this polypeptide chain is shown in Fig. 2, with the active site regions of several other coagulation proteins from bovine plasma. Twenty residues were identified in this peptide. The amino-terminal residue was valine and no other amino acids were detected. The active site serine, corresponding to serine 195 in chymotrypsin, appeared in position 17 of the peptide. The yield of this residue, however, was poor. For the remaining amino acids, the repetitive yields for the degradations were about 95% and over 80% of the expected quantities of the phenylthiohydantoins based on the weight of the lyophilised peptide. It is clear from these data that bovine factor XI contains an amino acid sequence which is characteristic of the active site regions of a number of plasma serine proteases. This sequence is also homologous to that found in pancreatic trypsin[6] and is consistent with the fact that factor XI_a has esterase activity towards benzoylarginyl ethyl ester[3] and participates in blood coagulation by splitting an arginyl valyl peptide bond in factor IX (P. Lindquist, K. Fujikawa and E. W. Davie, in preparation). A specificity toward basic amino acids is also consistent with the fact that residue 189 in factor XI is aspartic acid. This residue is in the bottom of the binding pocket in trypsin and forms an ion pair with an arginine or lysine residue in the substrate[7–12].

Bovine factor XI, like factor XII (Hageman factor), factor IX_a, factor X_a (activated Stuart factor) and thrombin, also contains an aspartic acid residue in position 194 next to serine 195. When trypsinogen and chymotrypsinogen are converted to enzymes, aspartic acid 194 forms an ion pair with the newly formed α-amino group of isoleucine which is generated during the activation reaction[13,14]. It seems likely that a similar mechanism may occur when factor XI is converted to a serine protease by limited proteolysis.

Factor XI contains 12 methionine residues per 124,000 g of glycoprotein[5]. This should yield 14 cyanogen bromide peptides for a molecule composed of two non-identical chains, or seven peptides for a molecule composed of two identical chains. Approximately 12 cyanogen bromide fractions were detected by gel filtration on Sephadex G-75 (Fig. 1). Examination of each of these peaks by sodium dodecyl sulphate polyacrylamide gel electrophoresis indicated, however, that many of these peaks were not homogeneous. Thus, the question as to whether factor XI is composed of two identical chains or two very similar chains remains to be answered.

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Depression: a new animal model sensitive to antidepressant treatments

A MAJOR problem in the search for new antidepressant drugs is the lack of animal models which both resemble depressive illness and are selectively sensitive to clinically effective antidepressant treatments. We have been working on a new behavioural model in the rat which attempts to meet these two requirements. The method is based on the observation that a rat, when forced to swim in a situation from which there is no escape, will, after an initial period of vigorous activity, eventually cease to move altogether making only those movements necessary to keep its head above water. We think that this characteristic and readily identifiable behavioural immobility indicates a state of despair in which the rat has learned that escape

Table 1 Effects of various psychotropic drugs on the total duration of immobility induced in rats forced to swim in a narrow cylinder for 5 min

Experimental treatment	Dose (mg kg^{-1} i.p.)	No. of rats per group	Duration of immobility (s) (mean ±s.e.)	*P* (difference from control)
Tricyclic antidepressants				
Imipramine HCl	Control	5	224.4±4.7	—
	7.5	5	188.2±14.6	N.S
	15	5	138.0±14.5	< 0.01
	30	5	132.4±25.9	< 0.01
Desimipramine HCl	Control	5	214.2±8.2	—
	2.5	5	220.8±22.8	NS
	5	5	202.2±14.3	NS
	10	5	174.8±16.8	NS
	20	5	98.8±17.6	< 0.01
Amitriptyline HCl	Control	10	245.4±8.0	—
	3.75	5	184.4±5.5	< 0.05
	7.5	10	189.7±18.9	< 0.05
	15.0	10	150.7±22.2	< 0.01
Monoamine oxidase inhibitor				
Nialamide HCl	Control	10	224.1±9.6	—
	40	10	231.2±20.3	NS
	80	5	127.4±29.6	NS
	100	5	83.4±43.0	< 0.01
ECS	Control	10	238.7±13.2	—
	ECS	10	166.0±24.7	< 0.05
Atypical antidepressants				
Iprindole HCl	Control	10	243.1±14.1	—
	7.5	5	268.4±11.1	NS
	15	5	237.8±15.9	NS
	30	5	229.4±21.3	NS
	40	5	155.6±24.8	< 0.01
	60	5	260.6±15.8	NS
Mianserin HCl	Control	5	210.6±21.1	—
	3.75	5	187.2±8.9	NS
	7.5	5	187.6±11.2	NS
	15	5	140.0±14.7	< 0.01
	30	5	147.2±11.5	< 0.05
	60	5	256.4±16.6	NS
Viloxazine HCl	Control	10	214.8±12.1	—
	7.5	5	207.6±30.2	NS
	12.5	5	205.6±12.9	NS
	15	5	202.6±21.1	NS
	25	5	176.0±23.2	NS
	30	5	171.0±15.1	NS
	50	5	144.8±15.3	< 0.05
Psychostimulants				
(+)-Amphetamine sulphate	Control	5	202.8±17.9	—
	0.75	5	175.0±9.2	NS
	1.5	5	85.4±19.4	< 0.01
	3.0	5	25.4±23.2	< 0.01
Caffeine	Control	5	219.6±20.7	—
	3.75	5	257.4±8.7	NS
	7.5	5	177.2±21.7	NS
	15	5	99.8±21.0	< 0.01
Anxiolytics				
Chlordiazepoxide HCl	Control	5	237.6±8.9	—
	2	5	237.2±16.0	NS
	4	5	225.6±9.6	NS
	8	5	234.6±9.4	NS
Diazepam	Control	5	208.0±10.7	—
	0.5	5	224.6±10.7	NS
	1	5	242.6±12.7	NS
	2	5	216.6±19.8	NS
	4	5	222.2±21.1	NS
Major tranquillisers				
Chlorpromazine HCl	Control	5	218.2±2.2	—
	0.75	5	220.6±11.8	NS
	1.5	5	224.0±18.1	NS
	3	5	263.0±8.2	< 0.05
Ro4-1284	Control	5	202.6±7.3	—
	1	5	271.2±12.6	< 0.01
	2	5	271.0±12.0	< 0.01
	4	5	257.8±15.9	< 0.01

The rats had previously received three intraperitoneal injections 24, 5 and 1 h before testing. The doses, expressed in terms of the salt, are those given at each injection. The drugs were dissolved or suspended in 0.9% NaCl and injected in a constant volume of 5 ml kg^{-1}. Differences from control values were assessed for statistical significance using the Dunnett test (two-tailed)[15].

is impossible and resigns itself to the experimental conditions. This hypothesis receives support from results presented below which indicate that immobility is reduced by different treatments known to be therapeutic in depression including three drugs, iprindole, mianserin and viloxazine which although clinically active[1–3] show little or no 'antidepressant' activity in the usual animal tests[4–6].

Naive male Sprague–Dawley (Charles River) rats weighing between 160 and 180 g are plunged individually into a vertical plexiglass cylinder (height 40 cm; diameter 18 cm) containing 15 cm of water maintained at 25 °C. After 15 min in the cylinder they are removed and allowed to dry for 15 min in a heated enclosure (32 °C) before being returned to their individual cages. This treatment produces long periods of immobility in the water (10–12 min total duration) and the rats on removal are mildly hypothermic (−3 °C) and are hypoactive for periods up to 30 min. 24 h later the rats are replaced in the cylinder and the total duration of immobility is measured during a 5-min test. Rats submitted to this procedure will remain immobile for 75% of the duration of the test (see Table 1) thus providing a suitable baseline for measuring the effects of drugs which decrease or even increase immobility.

Drugs and doses investigated are shown in Table 1. To optimise the pharmacological effect[7] and at the same time to imitate more closely clinical usage, we administered the drugs in a series of three intraperitoneal injections 24, 5 and 1 h before testing on day 2, the first injection being given immediately before replacing the animals in their cages on day 1. Electroconvulsive shock (ECS; 30 mA, 50 Hz, 1 s) was delivered through ear-clip electrodes according to the same time schedule as for drug injections.

Table 1 shows that all the antidepressants tested as well as ECS significantly reduced immobility. With the tricyclic compounds, particularly amitriptyline, this 'antidepressive' effect was accompanied by lowered muscle tonus and diminished motor activity outside the test situation suggesting sedation. Biphasic effects were observed with mianserin and iprindole: a reduction in immobility at lower doses followed at higher doses by a return to control levels and a marked loss of muscle tonus. Viloxazine decreased immobility without apparent sedative effects. The monoamine oxidase inhibitor, nialamide, reduced immobility but induced small repetitive body movements which were not observed with the tricyclic antidepressants; loss of muscle tonus was noted at 100 mg kg^{-1}. The two psychostimulants (+)-amphetamine and caffeine also reduced immobility but in contrast to the tricyclic compounds induced a marked hyperactivity; with (+)-amphetamine stereotyped head movements were observed at 1.5 and 3 mg kg^{-1}. Neither chlordiazepoxide nor diazepam affected the duration of immobility even at doses which produced noticeable ataxia. In contrast, both chlorpromazine and the reserpine-like compound RO4-1284 increased mobility; with RO4-1284 a ceiling effect was already observed at 1 mg kg^{-1}, a non-cataleptic dose.

We conclude from the results obtained that the immobility induced in these experiments reflects a state of lowered mood in the rat. Immobility was reduced by antidepressant drugs and ECS, was unaffected by anxiolytics, and was increased by drugs known to be capable of inducing depressive states in man[8,9]. The positive findings obtained with amphetamine and caffeine do not stand in contradiction to this conclusion as both compounds do possess some clinical antidepressant activity[10] and besides, the effects observed could be distinguished qualitatively from those of the non-stimulant antidepressants.

The results obtained with mianserin deserve comment because this compound, although clinically active[2], possesses a profile of pharmacological and biochemical activity which is almost the mirror image of that expected of a classical tricyclic antidepressant: it potentiates the effects of reserpine and antagonises the effects of amphetamine[11]. It does not inhibit the re-uptake of monoamines[12] and is moreover a potent blocker of central 5-hydroxytryptamine receptors[13]. Although providing no explanation for the mechanisms of action of mianserin, it is noteworthy that the present procedure, which is based on behavioural rather than biochemical concepts, is the first animal test model which would predict an antidepressant action for this compound. Furthermore the bimodal effect observed strikingly parallels clinical observations showing an optimal antidepressant effect at intermediate doses[14].

These positive findings, together with those obtained with the two other atypical compounds, iprindole and viloxazine, raise the intriguing possibility that the method described here might be capable of discovering new types of antidepressant agents hitherto undetectable using classical screening tests.

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Benzodiazepine receptors in rat brain

HIGH affinity binding of tritium labelled morphine and morphine-like drugs to membranes in brain homogenates[1–3] was a decisive advance in the characterisation of opiate receptors and the discovery of enkephalines and endorphines. We report here experiments which suggest that another important group of psychoactive drugs, the benzodiazepines, bind to specific receptors on the membranes of rat brain cells. This suggests that there may be an unknown endogenous neurotransmitter which is the natural ligand for the benzodiazepine receptor. The binding sites are distributed unevenly through the brain, and displacement potencies of benzodiazepines correlate with pharmacological effects predictive of anxiolytic activity.

Whole forebrains (excluding cerebellum and pons-medulla) of 150 g Wistar rats were homogenised gently in 20 volumes of ice-cold 0.32 M sucrose, centrifuged at 1,000*g* for 10 min and recentrifuged at 30,000*g* to give a crude P_2-synaptosomal fraction. The P_2-fraction was rehomogenised in an equal volume of hypotonic, 50 mM, Tris-HCl (*p*H 7.5). The binding assay consisted of 500 µl test drug solution and with ^{3}H-diazepam as the radioactive ligand, usually at 1.6 nM (^{3}H-diazepam (*N*-methyl-^{3}H) 14, 4 Ci mmol^{-1}; provided by Dr Willy Haefely, Hoffmann-La Roche). The mixture was preincubated for 5 min at 37 °C before addition of ^{3}H-diazepam followed by a 15 min additional incubation. The samples were cooled for 30 min in an ice bath and membranes with bound ^{3}H-diazepam were isolated on Whatman GF/C glassfibre filters. At the end of the incubation 10 ml of iced buffer (50 mM Tris-HCl, *p*H 7.4 was added, immediately before filtering, and the filter was washed with an additional 10 ml iced buffer and bound diazepam was estimated by conventional scintillation counting.

The amount of nonspecific binding, defined as binding in the presence of excess unlabelled ligand, was very low. Addition of diazepam at 3 μM to the binding assays displaced 91–93% of the total ^{3}H-diazepam binding. Specific ^{3}H-diazepam binding was saturable (Fig. 1) and maximal binding was obtained with about 50 nM ^{3}H-diazepam. A Scatchard analysis of the results in Fig. 1 yielded a single straight line in the concentration range below 50 nM, indicating the presence of a single binding site. The affinity constant, K_D (concentration of ^{3}H-diazepam giving half-maximal binding) was 2.6 nM and the total number of binding sites was 18 pmol per g original tissue. Hill plots (not shown) had a slope of 1.0–1.1 indicating absence of cooperativity.

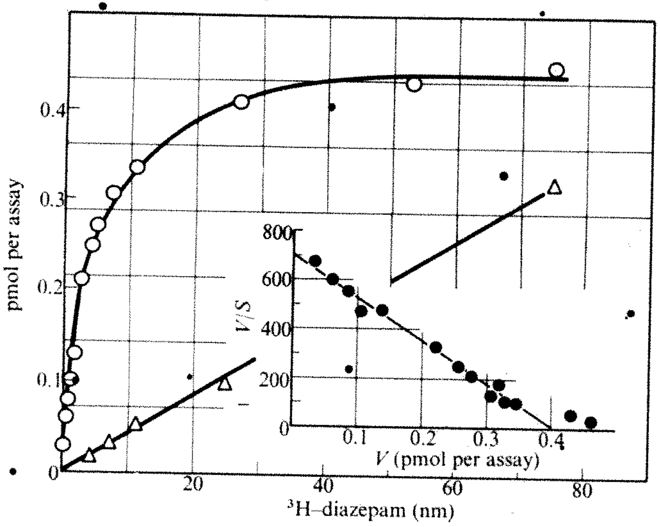

Fig. 1 Binding of ^{3}H-diazepam to membranes from 25 mg rat forebrain. ^{3}H-diazepam, 0.24–75 nM was incubated in triplicate with or without excess cold diazepam. Specific binding (○) is the total binding minus the binding which is not displaced by excess (3 μM) diazepam (●). Insert shows the same data transformed to Scatchard plots (V, specific binding in pmol per 25 mg tissue; S, ^{3}H-diazepam concentration in pM). The Scatchard plot indicates one binding site with affinity constant, $K_D = 2.6$ nM (reciprocal of the slope value) and maximal binding of 18 pmol per g original tissue (intercept on abscissae). The experiment was repeated, giving closely similar results. Counting efficiency was 44%.

Measurement of specific ^{3}H-diazepam binding (at 1.6 nM) in 10 rat brain regions showed an uneven regional distribution. The greatest binding was found in the frontal and occipital cortex where the binding was three to fourfold higher than the lowest binding in the pons-medulla. The hippocampus, which may be an important site of action for benzodiazepines in the limbic system[4], had intermediate binding levels.

The ^{3}H-diazepam binding site was highly selective for benzodiazepines. Twenty one benzodiazepines (Table 1) displaced ^{3}H-diazepam with IC_{50} values in the range of 3×10^{-9} to 10^{-4} M while some other 'minor tranquillisers', such as meprobamate, barbiturates and ethanol, were inactive at 0.1 mM. Several presumed neurotransmitters were tested as ^{3}H-diazepam displacers. None of these (acetylcholine, noradrenaline, dopamine, serotonin, GABA, L-glutamate or glycine, all at 0.1 mM) or their antagonists (phenoxybenzamine, propranolol, pimozide, clozapine, methysergide, bicuculline, picrotoxin or strychnine, all at 3×10^{-6} M) exhibited any displacement activity. Various other compounds, including etorphine, nalorphine and muscimol (all at 3×10^{-6} M) were also inactive.

Even though the binding of a compound may be saturable and show remarkable selective displacement properties it may not occur at meaningful recognition sites[5]. The diazepam receptor which we describe here, however, seems to be physiologically significant since it can be correlated to pharmacological activities. Efficacies in several pharmacological tests are available for most of the benzodiazepines used in the present study[6]. Excellent correlations ($P > 0.001$) were observed between ^{3}H-diazepam displacement potency on the one hand and cat muscle relaxant effect, impairment of mouse rotarod performance, inhibition of electric shock induced fighting in mice and antagonism of pentazol induced convulsions in mice, on the other. Weak correlation was found to taming of cynomolgus monkeys ($P = 0.01$, $r = 0.655$). Displacement of ^{3}H-diazepam binding correlates best with the cat muscle relaxant effect (Fig. 2). While the pharmacologial tests listed above are predictive of anxiolytic and anticonvulsant activity we found no correlation with the non-predictive conditioned avoidance tests (either shock increase or escape failures) in the rat.

Table 1 Inhibition of specific ^{3}H-diazepam binding (1.6 nM) to rat brain membranes by benzodiazepines

Compound*	IC_{50}(nM)†
RO 5–4023 (clonazepam)	5
RO 5–4200 (flunitrazepam)	5
Lorazepam	7
RO 5–3027	9.2
RO 5–3590	13.1
RO 5–6901 (flurazepam)	28
RO 5–2807 (diazepam)	34
RO 5–3059 (nitrazepam)	34
RO 5–3350 (bromazepam)	48
RO 6–6616 (chlorazepate)	62
RO 5–2904	76
Oxazepam	80
RO 5–2181	135
RO 5–4528	389
Chlordiazepoxide	1,072
RO 5–5807	4,487
RO 5–3785	5,686
RO 5–4556 (medazepam)	6,217
RO 5–3636	8,270
RO 5–4933	<30,000
RO 5–4864	163,522

Serial dilutions of benzodiazepines (in duplicate experiments) were added to the high affinity binding assay (see text).

*Chemical structures given in ref. 6.

†IC_{50}, concentration causing 50% inhibition of specific ^{3}H-diazepam binding.

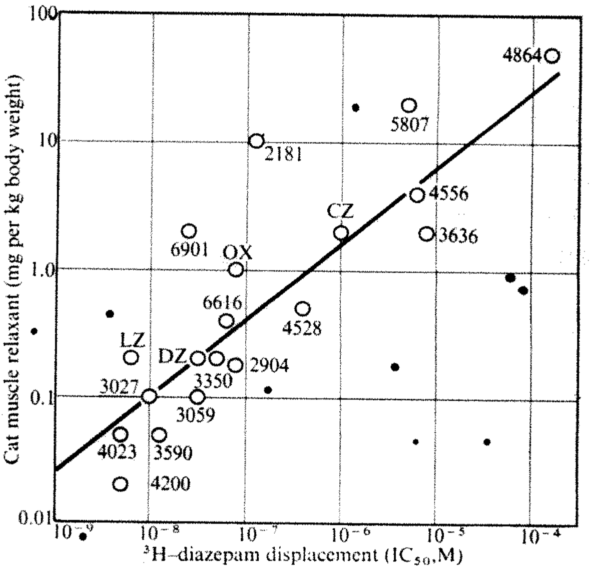

Fig. 2 Correlation between IC_{50} values for ^{3}H-diazepam displacement by 19 benzodiazepines (from Table 1) and cat muscle relaxant (oral dosage) effect. The best curve fit was assessed by linear regression analysis ($r = 0.891$; $P < 0.001$). Numbers on graph indicate RO code number, OX, oxazepam; DZ, diazepam; LZ, lorazepam; CZ, chlordiazepoxide.

In a comprehensive review, Randall *et al.*[6] concluded that the 'actions of benzodiazepines cannot be related in a direct manner to the action of neurotransmitters in the brain'. The proposal[7] that benzodiazepines act on glycine receptors seems now unlikely in view of electrophysiological data[8,9] and our finding that neither glycine nor its antagonist strychnine displace ^{3}H-diazepam binding. Further, the benzodiazepines were about 10,000 times more potent as displacers of ^{3}H-diazepam binding than ^{3}H-strychnine binding. And ^{3}H-diazepam does not apparently bind to GABA-receptors, since GABA itself, its potent agonist muscimol and its antagonists picrotoxin and bicuculline were completely inactive as ^{3}H-diazepam displacers. The failure of benzodiazepines to block ^{3}H-GABA binding to brain membranes[10] further indicates the lack of direct benzodiazepine interactions with GABA receptors. These results, however, are not inconsistent with the proposal that benzodiazepines exert their physiological effects through GABA[11], since such effects may be indirect (for example, benzodiazepines might facilitate GABA release).

Our findings strongly suggest that the brain possesses specific receptors for benzodiazepines which may mediate their pharmacological actions. The exact mechanisms by which benzodiazepines exert their characteristic pharmacological and clinical effects remain unknown. A search for a hitherto undiscovered endogenous transmitter substance, acting on the benzodiazepine receptor therefore seems worthwhile.

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Axotomy causes loss of muscarinic receptors and loss of synaptic contacts in the hypoglossal nucleus

MUSCARINIC receptors in the brain and ileum can be studied by means of the binding of radiolabelled cholinergic antagonists[1,2]. The basic criteria for receptor-specific labelling are that there should be a saturable component of binding and that the binding of the radiolabelled ligand should be inhibited by pharmacologically effective concentrations of drugs which are known to act at muscarinic receptors and not by drugs that act at different receptors. In addition, the saturable binding component should be restricted to tissues known from pharmacological experiments to contain muscarinic receptors. These criteria are satisfied by tritiated propylbenzilylcholine mustard (^{3}H-PrBCM)[3], a potent irreversible muscarinic antagonist the binding of which to rat brain sections can be demonstrated autoradiographically. The evidence which suggests that the bulk of tissue radioactivity is associated with muscarinic receptors is that in autoradiographs the regional variation of autoradiographic grain density parallels the intensity of specific ^{3}H-PrBCM binding observed by *in vitro* assay on brain homogenates from selected regions, and that in the homogenates 65–85% of the bound radioactivity is blocked by pretreatment with the classical muscarinic antagonist, atropine (10^{-6} M). In the forebrain the general features of the distribution of ^{3}H-PrBCM binding to thin sections correspond closely with the findings of Kuhar and Yamamura[4,5] who used the non-covalently bound antagonist ^{3}H-3-quinuclidinylbenzilate. We report here, however, that in the brain stem the hypoglossal nuclei are heavily labelled with ^{3}H-PrBCM. In light microscopic autoradiographs (Fig. 1*a*, left side) the grains are distributed throughout the neuropil of the hypoglossal nucleus. The cytoplasm and nuclei of the large hypoglossal neuronal somata (Fig. 1*b*) are unlabelled. The density of the silver grains falls abruptly at the borders of the hypoglossal neuropil. This clear demarcation of the labelled hypoglossal neuropil is abolished by atropine pretreatment (Fig. 1*c*).

The present results are based on a total of 48 adult female Wistar rats in which the hypoglossal nerve was cut on one side in the neck. In 30 animals the brains were prepared for light microscopic autoradiographic study of receptor binding, and in a further 18 animals the brains were prepared for an electron microscopic study of synapse numbers.

Several studies[6-9] of the effects of axotomy on the hypoglossal nucleus have shown that by 1 week after operation there is a loss of about 50% of the synapses on the hypoglossal motoneurones. If the hypoglossal axons are able to regenerate and re-establish neuromuscular contacts, synapses reappear in the hypoglossal nucleus, indicating that the synaptic 'disjunction' is reversible. We have examined the hypoglossal nucleus at various times after section of the hypoglossal nerve on one side, and the preliminary series of experiments indicated that two changes are detectable within 2 d after axotomy, and seem to have reached completion by 7–10 d after operation.

There was a marked decrease in the intensity of binding of ^{3}H-PrBCM. Since the nerve arises almost exclusively from the hypoglossal nucleus of the same side, and the two nuclei lie immediately adjacent to each other on either side of the midline, the opposite nucleus can be used as a control in the same coronal section. A representative indication of the magnitude of the changes may be seen from the grain counts taken from the sections shown in Fig. 1*a* and *c*. The numbers of silver grains per $10^{4}\ \mu m^{2}$ of section were 305±5 over the unoperated hypoglossal nucleus, 209±4 over the hypoglossal nucleus of the operated side (6 d after axotomy), and 123±3 over the hypoglossal nucleus of the unoperated side in the adjacent section (Fig. 1*c*) pretreated with atropine. Subtracting the atropine counts, this indicates that section of the hypoglossal nerve had caused a fall in the intensity of labelling of the hypoglossal nucleus of the operated side to 47% of its intensity on the unoperated side.

At the electron microscopic level, we counted the total numbers of synapses per unit area of section. As in the studies of Sumner[7] we found that there was an almost 50% reduction in the total numbers of synapses by 1 week after operation.

If the hypoglossal nerve is able to regenerate and re-establish contact with an appropriate peripheral target tissue, these changes are reversed. The hypoglossal motoneurones re-acquire a normal complement of afferent synapses[6-9] and our observations on the rats with the longer survival times showed that in those cases where the hypoglossal nerve had grown back, binding of ^{3}H-PrBCM in the hypoglossal neuropil had also recovered to normal levels. In some animals, the regenerating

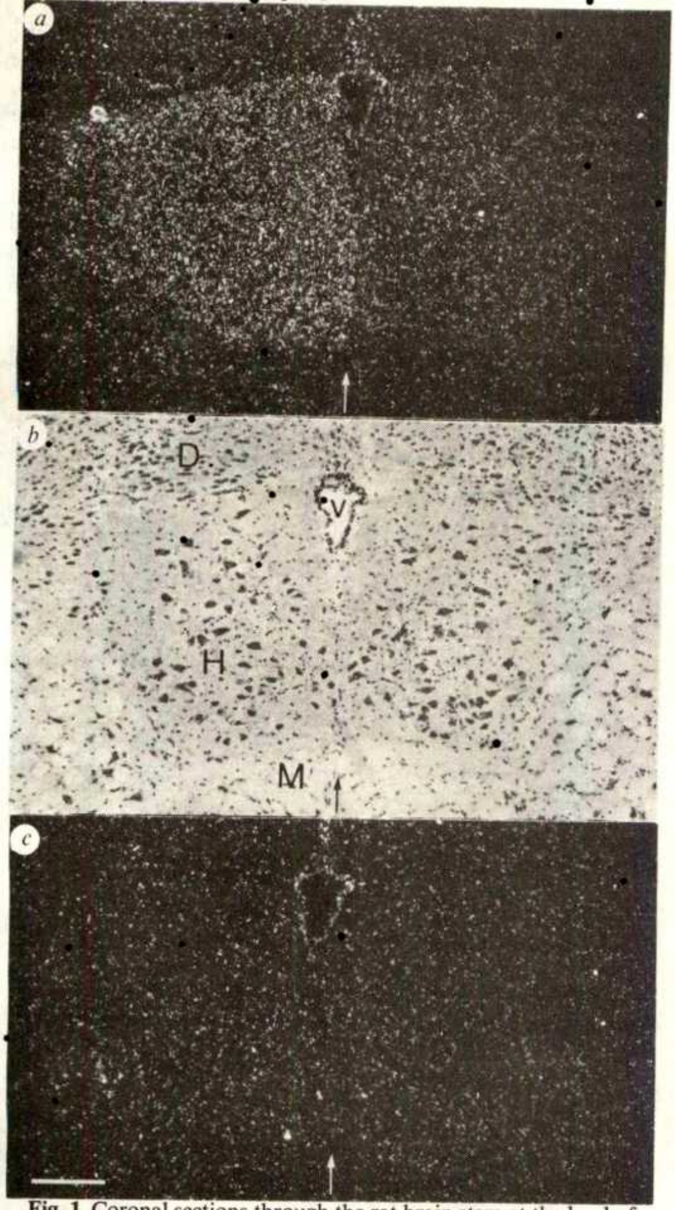

Fig. 1 Coronal sections through the rat brain stem at the level of the caudal medulla, where the hypoglossal nucleus has its major cross sectional area. The arrows show the midline. *a*, Dark field photomicrograph showing the intense deposition of autoradiographic silver grains over the hypoglossal nucleus on the left side of the picture (side of the intact hypoglossal nerve). Labelling falls abruptly at all the boundaries of the hypoglossal neuropil. On the opposite side, where the hypoglossal nerve had been sectioned 6 d previously (right side of the picture), the labelling is 47% of its intensity on the unoperated side. *b*, Bright field photomicrograph of the same section to show the cytoarchitectonic areas. H, hypoglossal nucleus, D, dorsal vagal nucleus, M, medial lemniscus, v, caudal ('closed') part of the fourth ventricle. *c*, Dark field photomicrograph of the adjacent section, which had been pretreated with 10^{-6} M atropine to abolish specific muscarinic antagonist binding. The hypoglossal nucleus shows no labelling above the level of the surrounding areas. Scale bar, 200 μm. For autoradiographic study of muscarinic receptor binding, the animals were anaesthetised with ether and perfused briefly with ice-cold Krebs–Henseleit medium. The brains were dissected out, frozen in solid carbon dioxide, and mounted on cryostat chucks. Coronal sections (12 μm thick) were cut at −15 °C, thaw-mounted on to gelatinised slides and pre-fixed in 0.1% glutaraldehyde in Krebs–Henseleit medium at 5 °C for 15 min. Pre-incubation was carried out for 15 min at 30 °C in Krebs–Henseleit medium with or without 10^{-6} M atropine, followed by addition of cyclised ^{3}H-PrBCM (40 Ci mmol^{-1}) to a final concentration of 2.4 nM for a further 15 min; these conditions lead to a more than 98% saturation of the receptors. The incubation was terminated by a quick wash in distilled water and post-fixation in Carnoy's fluid followed by several extensive washes in absolute alcohol. Slides were dipped in a 1:1 dilution of Ilford G5 nuclear emulsion, exposed at 6 °C for 14 d, developed in Ilford Phen-X, and counter-stained with cresyl fast violet.

proximal nerve stump had reconnected only incompletely, or not at all to the distal trunk. In these cases, the binding of ^{3}H-PrBCM had returned only in patches. Sumner[10] has shown that if the cut hypoglossal nerve is not allowed to re-establish peripheral contacts, there is no recovery of synapse numbers. Taken together, these observations indicate that in the case of the hypoglossal motoneurones axotomy causes both loss of synaptic contacts and loss of binding of ^{3}H-PrBCM, and both these changes are reversed if the axons regenerate appropriate terminal contacts, but not if they fail to do so.

In several sites in muscle[11,12] and nerve[13], experimental destruction of the presynaptic element causes postsynaptic cholinergic (nicotinic) receptors to increase in number, and a new population of receptors to appear outside their normally restricted subsynaptic location, indicating that the maintenance of normal receptor number and distribution depends on the presence of the presynaptic element. Although we know of no direct electrophysiological evidence for the occurrence of cholinergic transmission using postsynaptic muscarinic receptors in the hypoglossal nucleus, the occurrence of specific muscarinic antagonist binding strongly suggests the presence of such a mechanism. In the present experiments, damage to the postsynaptic neurone caused a decrease in the amount of binding of muscarinic antagonist, and a loss of synaptic contacts, and our preliminary results from a closely timed series of experiments suggest that the binding loss may preceed the loss of synapses. Our method of applying the PrBCM directly to the cut sections makes it likely that the decreased binding is due to a loss of receptors, and not to changes in the accessibility of the receptor (for example by the interposition of a glial barrier).

Taken together with the observation that axotomy causes a decrease in sensitivity to transmitter in several types of neurones (for example see refs 14 and 15), the findings in the hypoglossal nucleus suggest that there could be a converse regulatory relationship—namely that the presence of receptors may be necessary for the maintenance of the physical contact between the pre- and the postsynaptic elements.

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Specific long-lasting potentiation of synaptic transmission in hippocampal slices

SHORT bursts of tetanic stimulation (10–50 Hz for 10–15 s) give rise to long-lasting potentiation (LLP) of synaptic transmission in the intact hippocampal formation[1] and in hippocampal slices[2]. The potentiation may last several hours. Because of the time course and the small number of conditioning impulses required, the process may have general interest as a model for long-term plasticity in the nervous system. Studies of the mechanisms underlying LLP have been hampered, however, by lack of control with the size of the input volley and of data on membrane potential changes of the participating cells. Further, it is not known whether the potentiation is specific for the tetanised input. Schwartzkroin and Wester[2] found an unchanged antidromic field potential, and concluded that there were no general postsynaptic changes. Lynch *et al.*[3], however, found a reduction in glutamate sensitivity during the potentiated stage, and suggested that a reduction in excitability had taken place. We report here experiments on transverse hippocampal slices[4] of guinea pigs in which we have monitored the size of two independent afferent inputs, one of which is tetanised to produce LLP, leaving the other as a control line to check for non-specific changes in excitability. Furthermore, intracellular recordings support our suggestion that LLP is caused by a specific augmentation of transmitter release.

Figure 1 shows the location of a typical CA1 neurone with reference to the layers of the hippocampal slice, and the arrangement of the stimulating and recording electrodes used to study two independent pathways, one in the layer of basal dendrites (str. oriens) and the other in the layer of apical dendrites (str. radiatum). Both excite the CA1 pyramidal cells through fibres running parallel to the pyramidal layer. The fibres have *en passage* boutons which synaptically excite dendrites at the same level as the corresponding stimulating cathode (Fig. 1*b*). Field potentials were recorded with three electrodes, two placed at the same level as the two stimulating cathodes, and the third in the pyramidal layer, all three being placed on a line normal to the pyramidal layer, and recording from the same population of pyramidal neurones. In some experiments, the pyramidal electrode was used for intracellular recording.

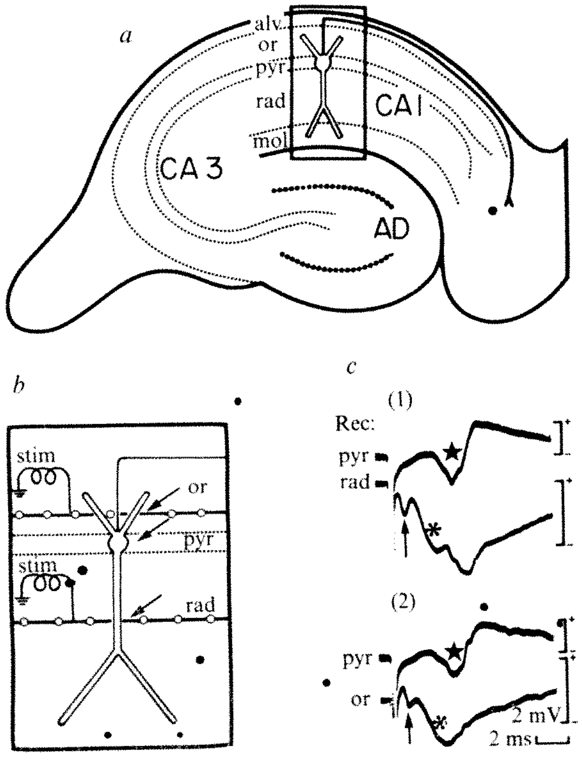

Fig. 1 *a*, Schematic drawing of the hippocampal slice. alv, alveus; or, str. oriens; pyr, str. pyramidale; rad, str. radiatum; mol, str. moleculare. *b*, Enlargement of box in *a* to show the arrangement of stimulating cathodes (stim) and recording electrodes (→) along two independent pathways, one in str. oriens and one in str. radiatum; *c*, Example of recorded potentials showing presynaptic volley (↑), extracellular EPSP (*), and population spike (★) in response to stimulation of a radiatum (1) and an oriens pathway (2). Left-hand labels indicate recording sites.

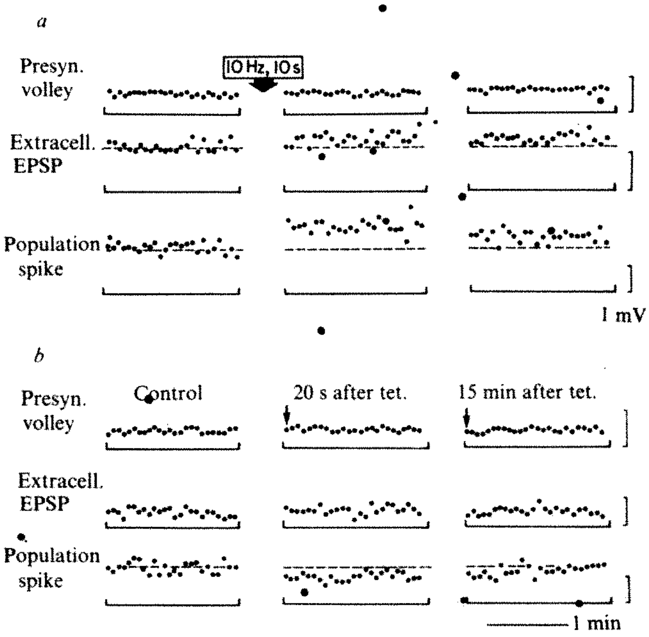

Fig. 2 Left-hand columns in *a* and *b* give control responses before a tetanus of 10 Hz for 10 s was delivered to the radiatum pathway. The middle column shows the responses obtained between 20 and 120 s and the right-hand column shows responses recorded between 15 and 16.7 min after the cessation of the tetanus. *a*, Increased amplitude of the extracellular EPSP in str. radiatum and of the population spike in the pyramidal layer in response to a constant radiatum fibre volley (0.25 Hz, 29 μA). A small increase of the presynaptic volley is seen in the right-hand plot. *b*, Lack of consistent changes of the amplitude of the presynaptic volley, extracellular EPSP recorded from str. oriens and of the population spike recorded from the pyramidal layer in response to stimulation of oriens fibres (0.25 Hz, 15 μA).

When a group of fibres was stimulated, the dendritic electrode at the same level recorded an initial diphasic deflection followed by a negative wave with an irregularly shaped peak (Fig. 1*c*). The initial diphasic deflection (↑) is taken as the sign of the presynaptic fibre volley for the following reasons: it was located strictly to the activated level, was linearly related to the EPSP amplitude, showed a conduction velocity of about 0.3 m s^{-1}, followed high-frequency stimulation, and was not changed by removal of Ca^{2+} from the bathing fluid (Andersen, Silfvenius, Sundberg and Wigström, unpublished observations). The following negative extracellular field potential (*) was directly correlated to intracellularly recorded EPSPs and is taken as the extracellular sign of EPSPs from a group of neighbouring cells. Finally, a population spike (★), which represents an envelope of unit discharges[5], was recorded from the pyramidal layer. The two pathways were stimulated alternately so that each line was tested every fourth second. We measured continuously the size of the presynaptic volley and the corresponding extracellular EPSP. Both may serve a measure of the input strength[6]. Further, we measured the size and peak latency of the population spike. After a control period of 15–30 min, a brief tetanisation was given to one of the pathways (10 Hz for 10 s or 50 Hz for 5 s).

Figure 2 shows synaptic transmission in the radiatum pathway to be greatly facilitated after tetanic stimulation of the same pathway, as indicated by an increased extracellular EPSP and a greatly enhanced population spike with reduced latency. Further, the probability of single unit discharges increased during the period of the enhanced extracellular EPSP. These effects subsided only gradually and could last > 1 h. It proved necessary to monitor the size of the presynaptic volley. In eight of 16 trials a transient increase of this deflection (< 10 min) occurred post-tetanically. Further, in some experiments, distinct and seemingly spontaneous changes of the presynaptic volley developed. Therefore, to assess the potentiation effects, input–output graphs were produced before and at various times after the tetanisation. Comparing the input–output curves taken before and after the tetanisation, a given afferent volley along the tetanised line gave rise to a larger extracellular EPSP (13 of 16 trials) and a larger (13/13) and shorter latency (12/13) population spike (Fig. 3). In none of the seven trials in which control line responses were measured, were there any observable changes in the latter, except a transitory and small change of the population spike, usually lasting < 3 min. The LLP, therefore, seems to be an input-specific effect.

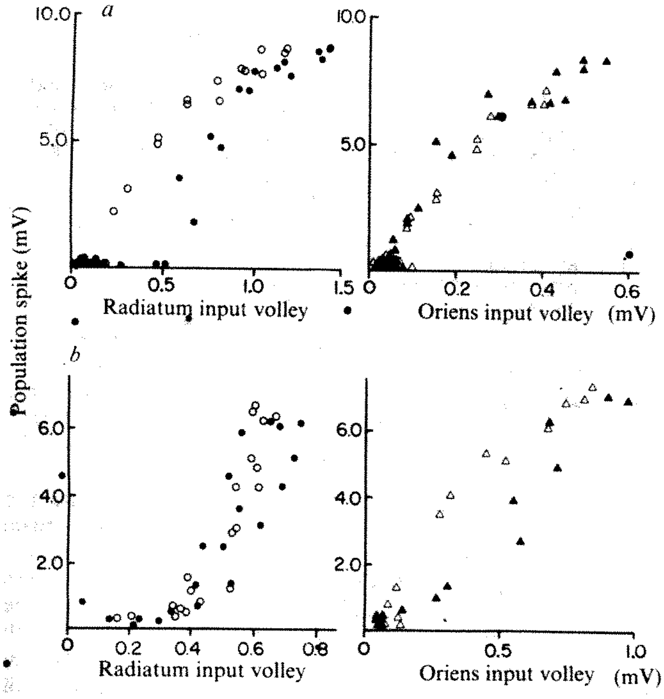

Fig. 3 Input–output graphs for radiatum line (left) and oriens line (right) in two slices. *a*, Slice in which tetanisation (50 Hz, 5 s) was given to str. radiatum. Responses to radiatum stimulation before (●) and after (○) tetanisation and to oriens stimulation before (▲) and after (△) tetanisation. *b*, Symbols as in (*a*) but a different slice and tetanisation (50 Hz, 5 s) now delivered to str. oriens.

We also compared the size of the presynaptic volley and the probability of discharge of single CA1 neurones. The input–output graphs changed after the tetanus in the same way as for the population spike. With intracellular recording the membrane potential, the size of the EPSP and the firing threshold of the spike were compared pre- and post-tetanically. In Fig. 4, three afferent volley sizes produce a larger EPSP after the tetanisation than before. The increased EPSP caused the spike to occur in response to a smaller input volley and with a shorter latency compared to the pre-tetanised values. The fact that the membrane potential did not change suggests that the increased EPSP is due to an augmented transmitter release. In these experiments we did not systematically test the spike threshold by injecting depolarising current pulses in the soma because this threshold was found to be the same as the threshold for synaptically activated spikes.

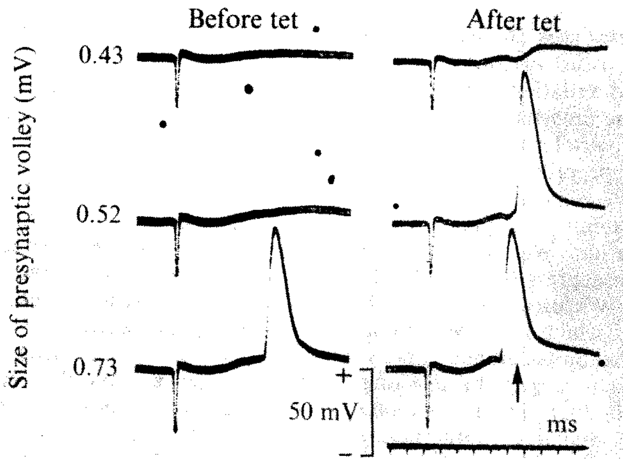

Fig. 4 Intracellular recording of responses to three differently sized radiatum fibre volleys. The peak-to-peak size of the volleys are given in mV on the left. Left-hand column were taken before, right-hand column 15 min after a 50 Hz, 5 s tetanus was delivered to the same radiatum fibres. After the tetanus a given input volley gives a larger and faster rising EPSP and increased probability of firing and a reduced latency. No significant change in the membrane potential occurred except during and shortly after the tetanus. Neither was there any change in the firing level of the action potential. The arrow indicates the latency of the control spike.

The claim for an input-specific process underlying the LLP rests on our observation of unchanged control line responses. Admittedly, small changes of these were seen, but an increase in excitability were as likely as a decrease, and both were unrelated to the tetanisation. Our failure to observe systematic changes in the control line responses makes it difficult to ascribe the reduced glutamate sensitivity seen during LLP to a general depression of post-synaptic excitability as done by Lynch *et al.*[3].

In conclusion, we believe the presented evidence suggests that short tetani of fibres having dendritic synapses on CA1 hippocampal pyramids lead to an input-specific and long-lasting potentiation (LLP) of synaptic transmission. The effect is hardly due to changes in the afferent volley since our method allows control of the latter. Because unwanted changes in the input volley can occur, we feel that experiments on LLP require adequate input control.

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Heterosynaptic depression: a postsynaptic correlate of long-term potentiation

BRIEF trains of electrical stimulation delivered to any one of several hippocampal pathways often result in immediate and long-lasting enhancement of subsequent postsynaptic responses elicited by that pathway[1–5]. The unusual characteristics of these effects suggest that theories of long-term potentiation (specifically changes in transmitter release by the presynaptic terminal) which have evolved from work on invertebrate and neuromuscular preparations may not be appropriate to explain synaptic plasticity in hippocampus. We report here the results of experiments intended to examine the postsynaptic consequences of repetitive stimulation. We found that potentiation of one afferent tended to depress the target cell's responses to a second test input.

We used the *in vitro* hippocampal slice technique, details of which have been published[6,7]. Briefly, rats were stunned, the hippocampus rapidly dissected free, and then sliced at right angles to its longitudinal axis on a Sorvall tissue chopper. The slices (500 μm thick) were then transferred to a small recording chamber of our design[7] and immersed in a slightly modified glucose–Ringer solution. All experiments were run at 33 °C.

Figure 1 shows the experimental design. The CA1 field was chosen for the analysis because it receives two different inputs to completely different dendritic subfields—namely, the apical and basal dendritic fields. Because of this anatomical arrangement, it is relatively easy to study the interaction of two spatially separate afferents on the same postsynaptic neurones. One bipolar stimulating electrode (twisted 62-μm wire) was placed in the trajectory of the Schaffer collateral-'apical' commissural projections to the stratum radiatum of the CA1 pyramidal cells. A second bipolar electrode was localised in the stratum oriens of the CA1, a zone which contains a massive commissural projection to the basal dendrites of the CA1 cells[8,9]. A recording micropipette was then guided by visual landmarks and physiological responses (cell spikes) into the pyramidal cell body layer (stratum pyramidale). The distances between stimulating and recording electrodes were typically 1–3.0 mm. A total of 28 slices was tested.

Low voltage stimulation to these pathways produced the responses characteristic of the laminated hippocampal projections—a monophasic negative-going potential in the dendritic zone innervated by the stimulated afferent and a monophasic positive potential in the stratum pyramidale. The response was similarly positive but considerably attenuated in the dendritic zone across the cell body layer from the stimulated afferent. As voltage was increased the 'dendritic' negative potential grew larger and a prominent sharp negative deflection appeared in the positive response recorded from the cell bodies. This negative potential is likely to be generated by the synchronous spike discharge of several pyramidal cells, as shown by Anderson *et al.*[10], and, following their terminology, we refer to it as a 'population spike'.

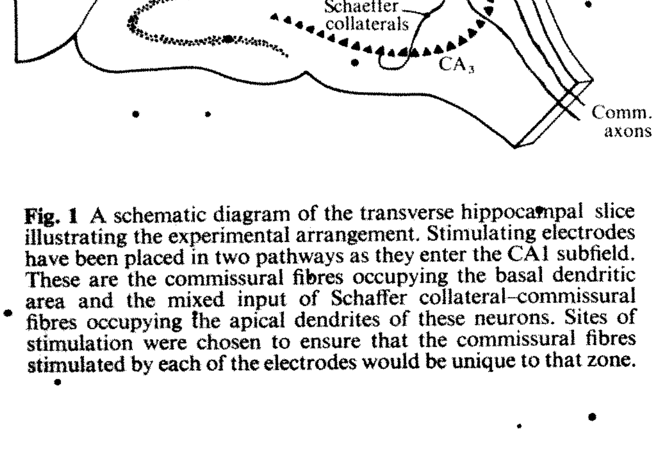

Fig. 1 A schematic diagram of the transverse hippocampal slice illustrating the experimental arrangement. Stimulating electrodes have been placed in two pathways as they enter the CA1 subfield. These are the commissural fibres occupying the basal dendritic area and the mixed input of Schaffer collateral–commissural fibres occupying the apical dendrites of these neurons. Sites of stimulation were chosen to ensure that the commissural fibres stimulated by each of the electrodes would be unique to that zone.

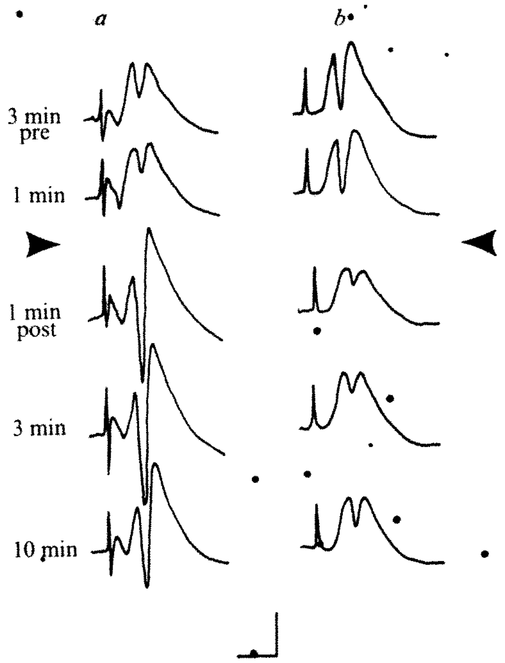

Fig. 2 An example of the depression seen in the commissural response (*b*) concommitant with post-tetanic potentiation of the Schaffer response (*a*). Note that all aspects of the commissural evoked response exhibit depression. The component most drastically reduced is the negative population spike, while the Schaffer population spike shows the greatest degree of potentiation following tetany. The potentiation stimulus consisted of a 15-Hz, 15-s train delivered (at test voltage) through the Schaffer electrode at the arrows. All records are an average of four responses; the test frequency was 0.2 Hz, pulse duration was 0.1 ms. Vertical scale, 1 mV; horizontal scale, 5 ms.

Following the placement of the three electrodes, the size of the responses evoked by the two electrodes was made approximately equal by adjustments of the stimulating voltages (usually 5–10 V). The voltages selected were adequate to generate a small (1 mV) population spike. The responses in both pathways were periodically tested with trains of four pulses presented at 0.2 Hz during a 5–10-min pretest. If these remained stable, a brief train (2–15 s) of 15-Hz stimulation was delivered to one of the two electrodes, then the frequency was returned to 0.2 Hz. In most experiments, the magnitude of the conditioning and test responses was sampled at 1-min intervals post-potentiation by averaging trains of four pulses given at 0.2 Hz. The potentiation train was delivered to the 'Schaffer' electrode in 13 slices and to the 'commissural' electrode in 15 cases.

During the stimulation train both the conditioning and test responses (that is, the responses to the electrodes which received the 15-Hz train and to the one which did not, respectively) were greatly augmented. After the termination of the train the two responses behaved quite differently. In 26 of 28 of the slices, the response elicited by the electrode which had delivered the high-frequency train was potentiated, particularly with regard to the population spike. This facilitation usually was evident within one or two pulses after the train and persisted for at least 15 min. Responses to stimulation in the non-potentiated pathway declined rapidly (over 15 s) towards baseline following repetitive stimulation of the other electrode, and in 22 of the cases entered a period of persistent depression (mean size of the population spikes tested at 2 min post-conditioning train was 68% of the control value ($P<0.001$) (Fig. 2 and see

Fig. 3). As was the case for potentiation, the depression was more evident in the population spike than in the slower field potentials.

Thirteen of the slices were tested for longer post-tetani periods and both potentiation and depression lasted for at least 15 min (Table 1). To test for effects longer than this it would be necessary to establish control procedures to insure that the status of the preparation remained stable for longer periods.

In these 13 slices we also delivered a tetanic train of stimulation to the depressed pathway, usually at 16 min after the first potentiation train. In all but one of these cases the depressed response showed marked potentiation, whereas in every case the previously potentiated responses were depressed (averages were 327% and 76% of the immediate pre-tetanus response 2 min following the reversal train).

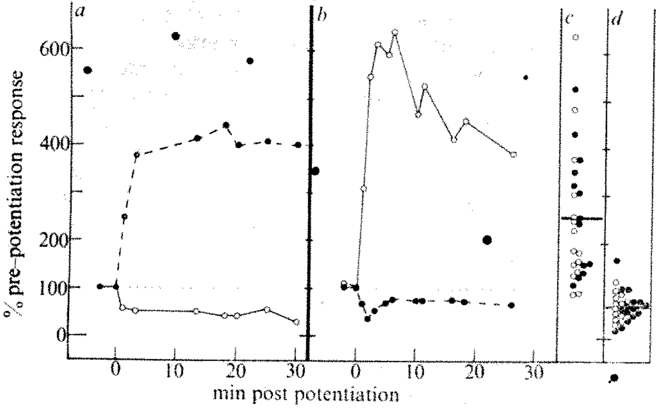

Fig. 3 Amplitude plot of the population spike evoked by both stimulating electrodes during 0.2-Hz 'test' stimulation. The figure shows the size of the population spikes both before and after a 15-Hz, 15-s train was delivered to either the Schaffer (*a*) or commissural (*b*) afferents at time 0 (different slices). *c* and *d* show potentiation and depression, respectively, of a group of slices whose responses were tested at 2 min after the potentiating train. Amplitudes are plotted as the percentage of the last non-potentiated response. Schaffer responses indicated by solid circles and commissural responses by open circles. Bars indicate means.

It seemed that, for most slices, long-term potentiation of one input to the pyramidal cells was accompanied by an unusually persistent depression of the response to remaining inputs. As the anatomical arrangements of the slice and the afferents used in this experiment are such as to make any axo-axonic interactions highly unlikely, our results suggest that brief trains of 10–15 Hz stimulation lead to two effects: (1) a local enhancement or potentiation of the driven afferent and (2) a generalised depression of the postsynaptic neurones.

Other lines of evidence also suggest that repetitive stimulation leads to some type of persistent and generalised depression of the postsynaptic cells. We have reported elsewhere[11] that long-term potentiation is accompanied by a persistent decrease in cell firing in response to glutamic acid applied by iontophoresis to the dendrites of the CA1 pyramidal cell.

Table 1 Comparison of population spike amplitude before and after tetanic stimulation

		Minutes post tetanus		
	Pre	5	10	15
Tetanised pathway	100%	390%	380%	332%
Control pathway	100%	74%	67%	73%
	$N = 17$	$N = 17$	$N = 17$	$N = 13$

All post-tetanic values were significantly different ($P < 0.05$) from immediate pre-tetanus values, as determined by a matched-pairs signed-ranks test.

In the absence of intracellular recordings we cannot dismiss the possibility that two intermingled sets of neurones are involved in the two effects, that the potentiation is localised to one set of cells and the depression to another. This seems unlikely, as it is quite evident from extracellular recordings that the same cells are driven by the two afferents used in our experiments. But whether we are considering opposing effects on a single neurone or in a small population of cells the present findings suggest that a kind of physiological modelling may go on in the hippocampus in which one afferent through repetitive firing gains at least a temporary priority at the expense of its neighbours. Finally, our results indicate that potentiation or depression of a pathway does not diminish the capacity of that system to undergo further plastic changes in response to subsequent episodes of repetitive stimulation.

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Ethanol reduces excitatory postsynaptic current duration at a crustacean neuromuscular junction

ETHANOL potentiates the postsynaptic voltage response to acetylcholine at mammalian and amphibian neuromuscular junctions[1–4], by prolonging the duration of the postsynaptic conductance change[5–7]. At these synapses, hyperpolarisation also increases the time constant of decay (τ) of end-plate currents[8–14]. Because of this voltage sensitivity, it was proposed[15] that the rate-limiting reaction in the decay of end-plate currents involves the relaxation of membrane proteins, which undergo a change in dipole moment normal to the field direction as they change conformation. The magnitude and direction of the change in dipole moment then determines the effect of electric field on α, the reaction rate. α ($=\tau^{-1}$) would be proportional to $\exp(V\mu)$ (ref. 15) where V is membrane potential and μ the dipole moment change normal to the field direction. It has been suggested that the prolonged decay of end-plate currents produced by ethanol is due to increase by ethanol of the dielectric constant of the environment of receptors[6], which lowers the rate of reactions involving a decrease in dipole moment[16] and hence increases τ. In some arthropods, including crayfish, crab and locust, there is evidence that glutamate acts as an excitatory transmitter at neuromuscular junctions[17,18]. Also, the decay of excitatory postsynaptic currents in crayfish[19] and locusts[20] has the opposite voltage sensitivity to that found at amphibian neuromuscular junctions: hyperpolarisation reduces τ, suggesting that the rate-limiting reaction in the decay of conductance at these 'glutamate' synapses could be associated with a change in dipole moment which is opposite to that seen at amphibian neuromuscular junctions. Ethanol could then have an opposite effect at these two types of synapse, and depress synaptic transmission at glutamate synapses. We report here results of our study of spontaneous miniature excitatory junctional currents (MEJCs) in the crab, which confirm this hypothesis.

We studied the opener muscle (abductor of the dactyl) of the walking legs of the crab (*Scylla serrata*). Dissected muscles were continuously perfused with crab solution containing 466 mM NaCl, 8 mM KCl, 20 mM $CaCl_2$, 12 mM $MgCl_2$ and 10 mM $NaHCO_3$, *p*H 7.45 (ref. 21). Temperature was controlled to within 0.5 °C in the range 10–22 °C. The time course of decay of MEJCs was determined by recording extracellular potentials with low resistance (0.2–1 MΩ) electrodes (filled with 1 M NaCl) which were located focally over junctional regions. In some experiments, intracellular microelectrodes (3 M KCl) were used simultaneously to record membrane potential. MEJCs, which occurred at a low frequency (sometimes $> 1\ \text{min}^{-1}$) were recorded (using a Neurograph-N3 digital transient recorder), displayed on an oscilloscope screen and photographed.

Although the shape and duration of MEJCs varied, two distinct types were recorded. The 'fast' MEJCs examined here had a rapid growth phase (0.2–0.5 ms), then a slower decay lasting 2–5 ms at 20 °C (Fig. 1*A*). The other, 'slow' MEJCs, not studied here, had a variable, slower growth phase followed by a much slower, non-exponential decay phase lasting < 50 ms. The decay of most of the fast MEJCs could be described by a single exponential function, as at amphibian neuromuscular junctions (Fig. 1*B*)[11,12,22]. Similar MEJCs recorded from crab neuromuscular junctions have been described previously[23,24].

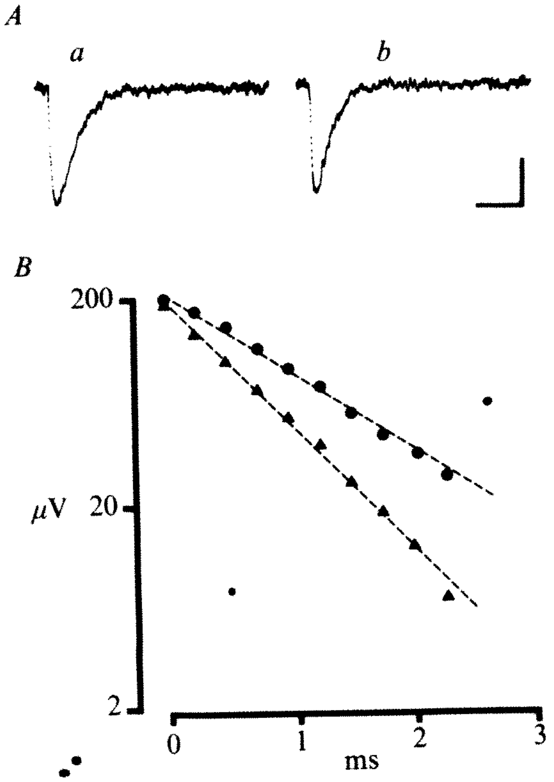

Fig. 1 Ethanol increases the rate of decay of MEJCs. *A*, MEJCs recorded in normal crab solution (*a*) and in the presence of 0.25 M ethanol (*b*). Calibrations: vertical, 100 μV; horizontal, 2 ms. *B*, semilogarithmic plot of the decay of MEJCs in control solution (●) and in 0.25 M ethanol (▲).

Recordings of these MEJCs were obtained in six preparations from cells with resting membrane potentials of −65 to −75 mV. The mean time constant of decay (τ) at 20 °C was 1.2±0.05 ms (mean±s.e.m.). The time constant of decay was temperature-sensitive, having a Q_{10} of approximately 2.0 in the temperature range 12–22 °C. The latter observation suggests that the decay of MEJCs is not rate-limited by diffusional loss of transmitter, a process which would be expected to have a lower Q_{10}. Ethanol (0.25 M) caused an obvious and reversible decrease in τ which is illustrated (trace (*b*) in Fig. 1*A*. The decay of MEJCs in ethanol remained exponential (Fig. 1*B*). In 0.25 M ethanol, τ was reduced from 1.2±0.05 to 0.5±0.02 ms, a 58% reduction. Lower concentrations of ethanol produced a smaller reduction in τ. The concentrations tested did not significantly change the membrane potential of the muscle fibres. Concentrations of ethanol, which at amphibian and mammalian neuromuscular junctions cause a significant increase in the duration of postsynaptic currents[5–7], produce a significant decrease in the duration of postsynaptic currents at crab neuromuscular junctions.

The results are consistent with the idea that effect of ethanol on the time course of postsynaptic currents is related to the change in dipole moment of the receptor during the reaction. The opposite voltage sensitivity of τ seen at glutamate-sensitive synapses[19,20] could reflect opposite changes in dipole moment, and this could be the explanation for the opposite effects of ethanol. There is no direct evidence for the same voltage sensitivity at the crab neuromuscular junction, but in preliminary experiments we observed that the rate of decay of MEJCs is slowed when the membrane is depolarised by increasing extracellular potassium concentration. It can be calculated[6] that an increase in dipole moment of about 40 debye in relaxing receptors would give the voltage sensitivity of τ seen at locust neuromuscular junctions[20]. This is similar in magnitude but in the opposite direction to the change in dipole moment calculated for amphibian neuromuscular junctions[6]. At toad neuromuscular junctions, 0.1 and 0.25 M ethanol increase τ by factors of 1.4 and 2.1, respectively[6]. It is interesting that at the crab neuromuscular junction we find that these concentrations of ethanol reduce τ by factors of 1.7 and 2.3, respectively. This agreement further supports the idea that ethanol could have a similar action at the two synapses, but that the sign of the change in dipole moment determines whether τ is increased or decreased. This explanation is not unique, and there are other possible ways in which ethanol could influence the rate of decay of synaptic currents differently at the two sites, for instance, by affecting differently the rate of dissociation of acetylcholine and glutamate from receptors if these reactions are rate-limiting.

The observation that ethanol can decrease τ at some synapses is interesting, as varied and selective actions of ethanol have been described previously in vertebrate and invertebrate nervous systems: ethanol potentiates cholinergic transmission at the amphibian and mammalian neuromuscular junction[1–4], and, conversely, has been shown to depress excitatory postsynaptic potentials in molluscan neurones[25,26]. It may be relevant that hyperpolarisation has been reported to increase the rate of decay of excitatory postsynaptic currents at some synapses in molluscan ganglia[27]. Ethanol also selectively depresses certain excitatory synapses in the central nervous system of goldfish without affecting other inhibitory and excitatory inputs[28], and depresses both inhibition and excitation of cat spinal motoneurones[29]. These depressant effects could arise from an increase in the rate of decay of postsynaptic currents, as seen at the crab neuromuscular junction. The reduction in amplitude of excitatory postsynaptic potentials caused by ethanol in molluscan neurones is associated with a reduction in their time to peak[26], probably due to an increase in the rate of decay of the underlying currents[30].

It is recognised that ethanol has anaesthetic properties. As many general anaesthetics reduce the time constant of decay of end-plate currents in amphibia and mammals, it has been suggested[31–33] that they may cause anaesthesia through a similar action in the central nervous system, that is, depression of synaptic transmission caused by a decrease in the duration of synaptic currents. A difficulty with this hypothesis has been that ethanol prolongs postsynaptic currents at the motor end-plate. We have now shown, however, that ethanol can reduce the duration of postsynaptic currents and hence depress transmission at a synapse where the transmitter is other than acetylcholine.

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Asymmetrical currents and sodium currents in Ranvier nodes exposed to DDT

THE non-linear component of the capacity current (asymmetrical 'displacement current') of nerve membranes has been thought[1–3] to result from the movement of intrinsic membrane charges gating the specific pathways for sodium ions ('Na channels'). This interpretation is partly based on the observation that the voltage-dependent relaxation time constants of the sodium activating mechanism roughly agree with those of the displacement currents. According to this hypothesis, a similar correlation should be demonstrated when the kinetics characteristics of the Na system are modified under controlled experimental conditions. In this respect, the insecticide DDT is a useful tool as it slows down the turning-off of the Na-permeability of the nodal membrane[4,5] without changing the rate of its turning-on. We have therefore compared the relaxation time constants of both the sodium current activation and the displacement current on DDT-treated Ranvier nodes. The results suggest that the intramembrane charge movement is not the sole transition involved in the gating process of the Na channels.

Experiments were carried out on voltage clamped myelinated fibres of the frog *Rana esculenta*. The delayed K current was abolished by tetraethylammonium (TEA, 10 mM) and by replacing the axoplasmic K content by caesium. The displacement currents (I_d), recorded in the presence of tetrodotoxin (TTX, 10^{-6}M), were obtained by subtracting the linear (leakage and capacity) current components in response to hyperpolarising voltage pulses from the responses to symmetrical depolarisations. Fifteen identical pulses were alternatively applied in both directions (starting from a holding potential of $E = -100$ mV) and the corresponding currents were digitised and averaged. DDT, first dissolved in ethanol, was diluted to 10^{-4}M in Ringer solution. Ethanol was present in the final DDT-test solution at a concentration of 2%, so was added at the same concentration, to the 'control solution' (Ringer + TEA + ethanol 2%). As a rule, the sodium current (I_{Na}) and the displacement currents (I_d) were first recorded in the reference solutions (control solution without and with TTX respectively). The nodes were then superfused with DDT-test solution for 20 min and the I_{Na} records were taken. Immediately afterwards, the TTX-control solution was re-admitted into the chamber and I_d was recorded. Taking advantage of the irreversibility of the DDT effects[5], this latter procedure permitted measurement of both I_{Na} and I_d after the same time of exposure to DDT. At the end of the experiment, the ability of the nodes to conduct I_{Na} and the persistence of the DDT effects were verified in the control solution.

In our experimental conditions, the effects of DDT on I_{Na} were in good agreement with those previously reported[4,5]. For reference, Na current records are presented in Fig. 1 *a* and *b*. They confirm that a long-lasting tail of Na current remains after repolarisation, indicating that in DDT-poisoned nodes a fraction of the Na channels remain activated for a long time. The time course of both the turning-on and the turning-off of the I_{Na} activation were plotted against time, assuming that I_{Na} is proportional to the m^3h product (Hodgkin–Huxley variables). The turning-on of the sodium activation is described by a single exponential function with a time-constant

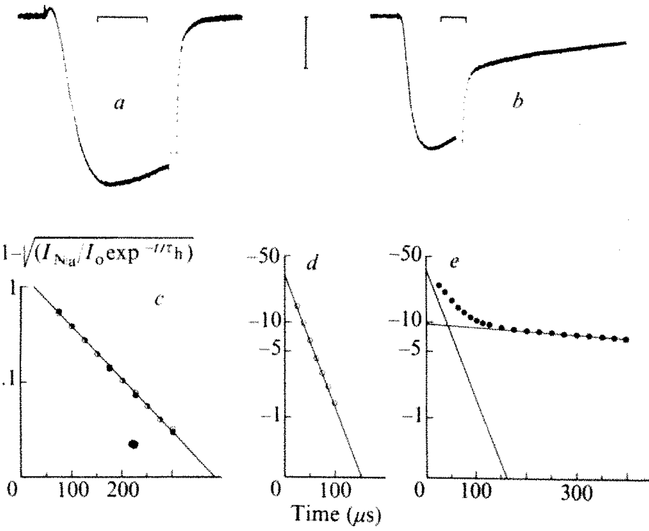

Fig. 1 Sodium current during 100 mV depolarising pulses from a holding potential of −100 mV. *a*, Before DDT and *b*, after exposure to DDT. Capacity and leakage currents were compensated analogically. Vertical and horizontal bars represent 5 nA and 200 µs respectively. Note the large tail of inward current in (*b*). The difference in the Na-current peak amplitude in (*a*) and (*b*) was not regularly observed. Graphs *c*, *d*, *e* are semilogarithmic plots obtained from the current records (*a*) and (*b*). At the onset of the depolarisation (*c*), before DDT (○) and after DDT (●). At repolarisation to the holding level, before DDT (*d*), after DDT (*e*). The straight lines in (*b*) represent the two exponential components giving the best fit to experimental points. Time constants of the single exponential functions: $\tau_m = 85$ µs (*c*); $\tau Na_{off} = 31$ µs (*d*); $\tau Na_{off,1} = 32$ µs (fast component in *e*); $\tau Na_{off,2} = 1390$ µs (slow component in *e*). Temperature 12 °C.

$\tau_m = 85$ µs which is unaffected by DDT (Fig. 1*c*). Under normal conditions (Fig. 1*d*) the turning-off can also be described by a single exponential with another time constant $\tau Na_{off} = 31$ µs. When DDT is added to the external medium (Fig. 1*e*), the tail of Na current is fitted by the sum of two exponentials: a fast one ($\tau Na_{off,1} = 32$ µs) very similar to that of Fig. 1*d*, and a much slower one ($\tau Na_{off,2} = 1390$ µs). This indicates that a fraction of the channels is unaffected by DDT whereas the rate of turning-off of the other fraction is nearly 45 times slower. From the extrapolated values of I_{Na} to time zero (t_0) in Fig. 1*e*, it may be deduced that, at the end of the depolarising pulse, 21.4% of the channels were affected in this experiment.

Figures 2 *a* and *b* represent the displacement currents obtained from the same fibre and at the same membrane potential as in Fig. 1. The analysis of these currents reveals that DDT does not affect the on-response of the displacement current (Fig. 2*c*) as expected from the results presented in Fig. 1*c*. However, the time constant $\tau d_{on} = 113\ \mu s$ is 1.3 larger than τ_m. Furthermore, the amount of displaced charges $[Q_{on} = I_{d,to}\,\tau d_{on}\,(1-e^{-t/\tau d_{on}})]$ is estimated to be 71 fC and is unaffected by DDT. In the absence of this agent (Fig. 2*d*) the time constant τd_{off} of the displacement current at the repolarisation is 42 μs (that is, 1.3 times larger than the corresponding Na_{off}). The amount of transferred charges ($Q_{off} = I_{d,to} \times \tau d_{off} = 55$ fC) is smaller than that transferred at the onset of the depolarisation. After exposure to DDT (Fig. 2*e*), two exponential components can be distinguished in the I_d off-response: a fast one ($\tau d_{off,1} = 42\ \mu s$), similar to that obtained from the control experiment, and a second one, markedly slower, with a time constant $\tau d_{off,2} = 190\ \mu s$. This second component is supposed to correspond to the fraction of charges affected by DDT. The best fit to the averaged points in the record of Fig. 2*b* leads to an estimate of the fractions of transferred charges ($Q_{off\infty,1}$ and $Q_{off\infty,2}$) of 28 fC (unaffected) and 27 fC (affected by DDT) respectively. The sum of both (55 fC) is identical to the amount of charges displaced at repolarisation under normal conditions.

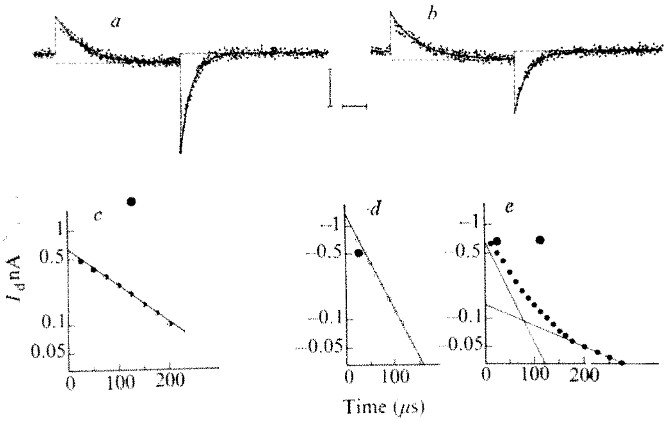

Fig. 2 Averaged displacement currents obtained from the same fibre as in Fig. 1 in response to 100 mV depolarising and hyperpolarising pulses from a holding potential of −100 mV. *a*, Before DDT and *b*, after DDT. Vertical and horizontal calibrations: 0.5 nA and 100 μs. The lines through the points represent the mean values taken as reference for the analysis presented in (*c*), (*d*) and (*e*). The small inward current component during the pulses is probably due to non-linearity of the leakage current between $E = 0$ and $E = -200$ mV or to discrete anodal breakdown during the hyperpolarising pulses; for this reason the zero-current reference for the on-response was taken at the end of the pulses. Graphs *c*, *d* and *e* are semilogarithmic plots of the I_d on (*c*) and off (*d*, *e*) responses, before DDT (○) and after DDT (●). The straight lines in (*e*) represent the two exponential functions: $\tau d_{on} = 113\ \mu s$ (*c*); $\tau d_{off} = 42\ \mu s$ (*d*); $\tau d_{off,1} = 42\ \mu s$ (fast component in *e*); $\tau d_{off,2} = 190\ \mu s$ (slow component in *e*). Estimated amount of displaced charges: $Q_{on} = 71$ fC (*c*); $Q_{off} = 55.3$ fC (*d*); $Q_{off\infty,1} = 28$ fc (fast component in *e*); $Q_{off\infty,2} = 27.2$ fC (slow component in *e*). Temperature 12 °C.

From a qualitative point of view, the effects of DDT on the displacement currents are those expected from the analysis of the sodium current, in particular when considering that DDT leaves the on-responses unchanged while the off-responses are altered. Thus, our results support the idea that the displacement current reflects the movement of charges involved in the activation process of the Na-conductance. Moreover, it appears that DDT acts only on charges previously displaced by depolarisation and at the time of reversion to their resting state (or position).

The fact that the fraction of charges affected by DDT (50% of the total amount in the experiment reported here) is larger than the fraction of affected channels (21%) is consistent with the assumption that at least two independent charges are involved in the gating subunit of each channel. If one assumes two particles per channel, only the channels in which both charged particles are affected by DDT will close slowly; those in which only one particle is affected will close at the same rate as intact channels.

A striking result is the observation that DDT increases the relaxation time constant of the I_d off-response by a factor of only 4.5 whereas it enhances that of the Na current by a factor of about 45. This indicates that the channels remain open for an appreciable time after the reversion of the gating charges to their resting configuration. This may be due to a specific effect of DDT. Disagreements between τd_{off} and τNa_{off} have been also observed, however, in squid axons submitted to external solutions made with heavy water[6]. Furthermore, discrepancies between the voltage dependence of the m-process and the displacement current have been reported for the squid axon[6] as well as for the Ranvier node[7] under normal conditions. There is thus good reason to suppose that DDT exaggerates the relaxation time of a further transition[6,8], complementary to the charge displacement and necessary for the closing of the Na channels.

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Effect of transplantation of pancreas on development of hypothalamic obesity

HYPOTHALAMIC obesity is a syndrome of hyperphagia and obesity that follows destruction of the ventromedial region of the hypothalamus. This syndrome is associated with loss of normal nocturnal rise in food intake and with several endocrine changes of which increased concentrations of insulin are the most important. It remains to be settled, however, whether the hyperphagia or hyperinsulinaemia is the primary factor in the development of hypothalamic obesity. The aim of the present experiment was to distinguish between these possibilities by freeing the insulin-secreting islets of Langerhans from neural control. This was done by implanting foetal pancreatic tissue under the kidney capsule of diabetic rats. This experiment prevented the characteristic hyperphagia and obesity seen in the control animals with normally innervated pancreatic tissue.

Obesity can be produced by several experimental procedures[1]. Among the most widely studied is the one due to damage to the ventromedial region of the hypothalamus[2,3]. For many years, hypothalamic obesity was ascribed to the destruction of a 'satiety centre' which was thought to coincide anatomically with the ventromedial nucleus of the hypothalamus[4]. Ablation of this 'satiety centre' was believed to remove the inhibitory influences from a primary feeding centre in the lateral hypothalamus and allow excess feeding.

Recent investigations have suggested, however, that this interpretation may be incorrect[5,6], and alternative hypotheses have been proposed. One of these suggests that the increase in food intake and obesity are both secondary to the rise in insulin levels which follow ventromedial hypothalamic (VMH) injury. The present studies were designed to critically examine this hypothesis.

An increase in the concentration of serum insulin after a VMH lesion is among the earliest observed changes[7,8], and occurs even when the food intake is limited and the increase in body weight is prevented[9,10]. If the increase in insulin is inhibited by destroying the β cells in the pancreas which produce insulin, the effects of a ventromedial hypothalamic lesion on food intake and body weight are abolished or largely reduced[11,12]. In addition, it is well known that injections of insulin will increase food intake and body weight[13]. This constellation of findings suggests an important role for insulin, but leaves unsettled the mechanism for its increase. Two possible hypotheses may be advanced. One is humoral, postulating that a 'hypothalamic factor' is released into the circulation and directly stimulates the release of insulin[14,15]. The other hypothesis proposes a neural mechanism[16]. A critical test of these two hypotheses might result from placing hypothalamic lesions in rats whose pancreatic islets were no longer under the neural influences of the pancreatic bed.

Female rats of the highly inbred Lewis strain weighing approximately 200 g were injected with streptozotocin (65 mg kg^{-1}). Four to seven days later, the diabetic rats (overnight fasting blood glucose levels >250 mg dl^{-1}) received transplants of pancreatic tissue from six foetuses which averaged 16–17 d old[17]. These transplants were placed beneath the renal capsule and the animals were observed for 4 weeks. The rats with effective transplants showed a gradual decline in serum and urine glucose and the insulin requirement disappeared. After 3 weeks of normal serum glucose, the recovered rats were tested for the function of the transplanted pancreatic tissue by giving them an intraperitoneal challenge with glucose (1 g kg^{-1}). The increase in serum glucose was similar in transplanted and control animals. Bilateral ventromedial hypothalamic lesions or sham-operations were introduced in the transplanted and control animals. Bilateral ventromedial hypothalamic lesions were produced using previously described coordinates[18] (at the Bregma anteriorly; ±0.7 mm lateral to midsagital line; and 1.0 mm above the base of the skull). Six of the eight control (non-transplanted) rats and five of the seven rats with pancreatic transplants had adequate hypothalamic lesions as confirmed by histological examination of stained sections of the brain. Data on body weight, the Lee index for body fatness at the final week and food intake measured two weeks after VMH lesions were compared using an analysis of variance with multiple range comparisons.

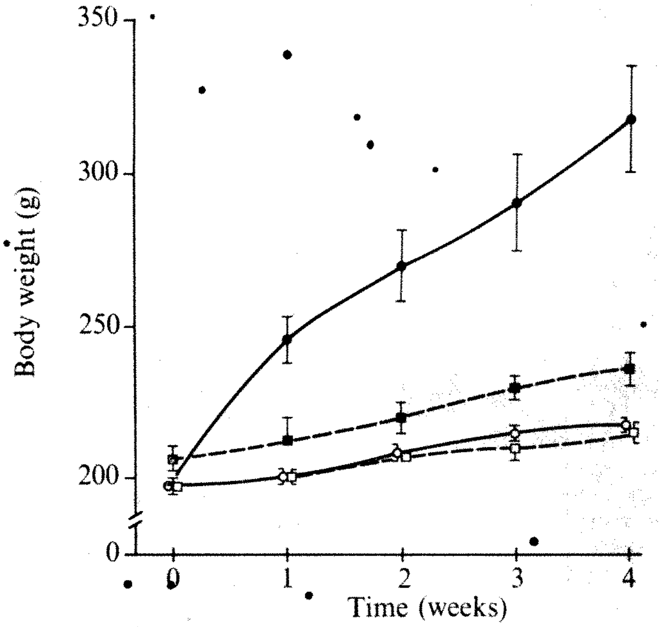

Fig. 1 Body weight of transplanted (■, □) and intact rats (●, ○). Body weight was followed for 4 weeks after VMH (●, ■). ○, □, Sham-operated. The increase in the lesioned rats without pancreatic transplants was statistically significant. The rats with pancreatic transplants and VMH lesions gained slightly, but not significantly more weight than the sham-transplanted animals. All data are presented as the mean ± s.e.m.

Body weight rose an average of 120 g in the lesioned rats with normal pancreatic tissue (Fig. 1) and reached an average of 319 g 4 weeks after the VMH lesions. The two groups of sham-operated rats (both with and without transplanted pancreatic tissue), showed a significantly smaller rise in weight than the VMH-lesioned rats with normal pancreatic tissue. There were no significant differences in the weight gain between VMH-transplanted rats and sham-operated animals. The Lee index, when the animals were killed, was used to measure fatness. It showed a significant increase in body fat in the control rats with VMH lesions, but no differences in body fat in the other groups, including the lesioned rats with transplants (Table 1). Food intake increased 71% in the lesioned rats with the normal pancreases but only 23% in the lesioned rats with transplanted tissue. The diurnal pattern of food intake was also measured during the third week after VMH operation. The ratio of food intake between 0900 and 1800, and between 1800 and 0900, was 3.3/14.5 and 3.1/14.3 for the two sham-lesioned groups. In the VMH-lesioned rats with normal pancreas, this ratio was 10.1/18.9. In the VMH-lesioned rats with transplants, the ratio was 6.0/14.9.

The results of the present experiment show that: removal of the pancreas from its usual neural influences markedly reduced the effect of ventromedial hypothalamic lesions on body weight and food intake; and that release of a humoral factor directly from the hypothalamus is not a sufficient

Table 1 Food intake and fatness of rats with transplanted pancreatic tissue, with or without hypothalamic lesions

Group	Animals	VMH	Operation pancreatic transplant	Food intake† ($g\ d^{-1}$)	P	Lee index*	P
1	6	+	–	29.3±0.8	<0.001	32.7±0.36	<0.01
2	6	–	–	17.1±0.4	<0.01	28.8±0.18	N.S.
3	5	+	+	20.5±0.5	<0.01	29.6±0.17	N.S.
4	5	–	+	16.7±0.4		29.2±0.14	

*Lee index $= \dfrac{(\text{body weight (g)})^{1/3}}{\text{naso-anal length (m)}}$; value at termination of experiment.

†Food intake was measured during the second week after VMH operation.

explanation for the high food intake and weight gain in rats with VMH lesions.

The vagus and sympathetic nerves both provide neural connections to the pancreas. The finding of gastric hypermotility and hyperacidity in rats with hypothalamic obesity suggests that the firing rate of the vagus nerve may be enhanced either directly or indirectly after VMH injury[19]. Further support for a role of the vagus nerve was provided by Powley and Opsahl who showed that vagotomy reversed the hypothalamic obesity[16] and by the fact that stimulation of the vagus increases insulin secretion[20,21]. The finding of a correlation between the level of acid in the stomach and the concentration of insulin in the serum of rats which had VMH lesions and varying degrees of vagotomy also suggest that the enhanced activity of the vagus nerve plays an important role in the syndrome of hypothalamic obesity[18].

The sympathetic nerves are also known to modulate insulin secretion from the pancreas[22,23]. Whether lesions in the ventromedial hypothalamus modify both components of the autonomic nervous system or only one remains to be established. What seems clearly demonstrated by the present experiment is that primary neural innervation of the pancreas plays a central role in the development of the increased food intake and obesity that follow ventromedial hypothalamic injury.

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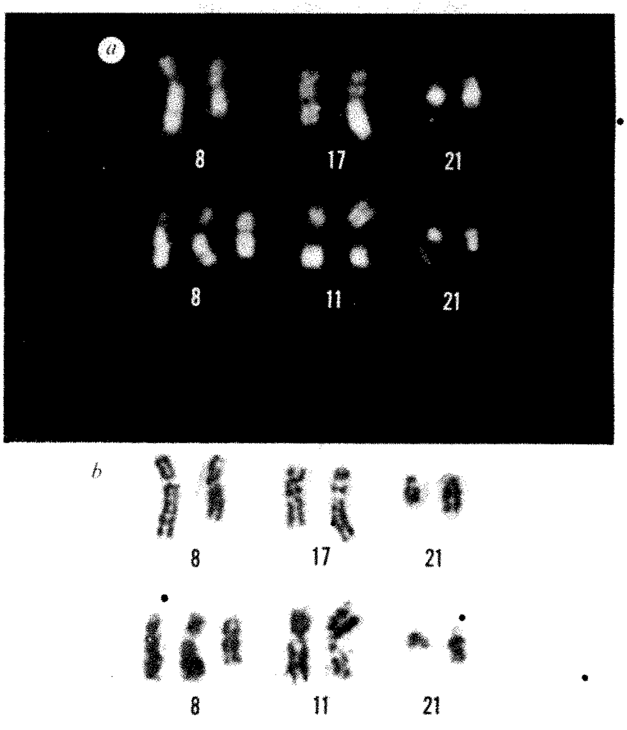
Comparable complex rearrangements involving 8;21 and 9;22 translocations in leukaemia

FOR many years chromosomal abnormalities in tumours seemed to be so variable that it seemed most reasonable to view them as epiphenomena. But banding techniques have revealed non-random chromosomal abnormalities in several human and mammalian malignancies. The observation of relatively consistent translocations in several types of leukaemia and lymphoma lends credence to Boveri's hypothesis[1] that chromosomal alterations are among the fundamental changes that result in malignancy. For example, the Philadelphia (Ph^1) translocation[2] is observed in 85–90% of patients with chronic myelogenous leukaemia (CML), and the 8;21 translocation is observed in 10–15% of patients with acute myelocytic leukaemia (AML)[3,4]. We report here that, when more complex structural changes occur, comparable patterns of rearrangements are found for both the 9;22 and the 8;21 translocations. This provides further support for Boveri's hypothesis.

The common rearrangement in AML involves a translocation of about one-third of the long arm of chromosome no. 8 to the end of no. 21 [t(8;21)(q22;q22)] (ref. 3). This rearrangement is often associated with loss of a sex chromosome, an X in female or a Y in male patients, which is otherwise a very rare occurrence in acute leukaemia. Whereas the original report[3] and subsequent confirmations[4] identified rearrangements involving only nos 8 and 21, we observed a variant translocation in a female patient with AML, in whom the translocation from no. 8 involved the end of the long arm of no. 17, and in whom there was an associated loss of an X chromosome (Fig. 1)[5]. Analysis with fluorescence revealed this abnormality in 22 of 26 cells (85%) obtained from the initial pretherapy sample and in 5 of 10 cells (50%) in a sample obtained 2 weeks later, while the patient was receiving chemotherapy.

A second female patient with a variant has been identified; the translocation from 8q involved the end of the short arm of no. 11 without loss of one X, but with the addition of one no. 8. In this patient, one no. 21 appeared larger than normal because of the presence of a narrow dull band of fluorescent material, presumably band 11p15 [47,XX,+8,t(8;11;21)(q22;p15;q22)] (Fig. 1). The complex

Fig. 1 Partial karyotype of cells from two patients. *a*, Stained with quinacrine; *b*, stained with Giemsa. Top row, chromosome nos 8, 17 and 21, showing the deletion of 8q22, transfer of this material to 17q23?, and the additional material on 21q presumably representing the end of 17q(q23→qter). Bottom row, chromosome nos 8, 11 and 21, showing the presence of two normal chromosomes no. 8 and the deletion of the third at 8q22, transfer of this material to 11p15, and the additional band on 21q presumably representing 11p15→pter.



translocation was seen in 4 of 18 cells (22%) in the pretreatment sample and in 13 of 14 cells (93%) in the sample obtained 10 d later.

Re-examination of the previous patient with the 8;17 translocation showed an analogous aberration of no. 21, compatible with a translocation from 17q (band 23?); the corrected karyotype should be 45,X,t(8;17;21)(q22;23?;q22). The break points in nos 8 and 21 in these two cases were similar to those in the standard 8;21 translocation.

The relevance of these observations to the role of chromosomal changes in leukaemia becomes apparent when one considers variant translocations in patients with Ph^1 positive CML. In most of these patients, a portion of the long arm of no. 22 (Ph^1 chromosome) is translocated to the end of the long arm of no. 9; this type of translocation was observed in 413 of 440 patients whose cells have been examined with banding[6]. Of the 27 cases with other rearrangements, 15 had translocations affecting three chromosomes; in each instance, two of the three chromosomes involved were nos 9 and 22, with the standard break points. Most of the remaining patients showed a translocation between no. 22 and some chromosome other than no. 9. Thus a parallel exists between the chromosomal changes in CML and in AML. In each case, two chromosomes are preferentially involved in a translocation; in some cases three chromosomes are affected, two of which are those involved in the typical translocation.

The mechanism producing such specific chromosomal rearrangements remains an enigma. Whether it is related to proximity in the nucleus or to homologous sequences of DNA, or whether it is the result of some selective advantage, is unknown. The observation of similar 'rules' governing two different translocations in two different types of leukaemia suggests that these changes are subject to precise, although as yet undetermined controls, and lends support to Boveri's hypothesis that chromosomal changes are among the fundamental alterations associated with malignancy.

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Derepression of *E. coli trp* operon on interfamilial transfer

THE P-group R factor RP4 originally isolated from *Pseudomonas aeruginosa* can be transferred to many Gram-negative bacteria[1–3]. In spite of its large molecular weight, RP4 has only one site susceptible to each of *Eco*RI (ref. 4), *Hind*III (ref. 5) and *Bam*NI (data not shown) restriction endonucleases. We have constructed an RP4-*trp* plasmid consisting of RP4 DNA and the complete tryptophan operon of *E. coli* obtained from bacteriophage λ*trpE-A*$_{60\text{-}3}$ *in vitro*[6]. We reported that the tryptophan synthetase activity in the strain carrying RP4-*trp* plasmid was repressed by exogenous tryptophan[7]. Manson and Yanofsky have reported that the *E. coli trp* operon on the colVB plasmid was similarly regulated in other enteric species[8]. We show here that the RP4-*trip* plasmid in *E. coli* can be transferred by conjugation to *Pseudomonas aeruginosa* which belongs to a different family from *E. coli*, converting *Pseudomonas* Trp$^-$ phenotype to Trp$^+$. The levels of tryptophan-synthesising enzymes in *P. aeruginosa* carrying RP4-*trp* plasmid are much higher than those in the wild strain, and they are not controlled by the repression system in *P. aeruginosa* cells.

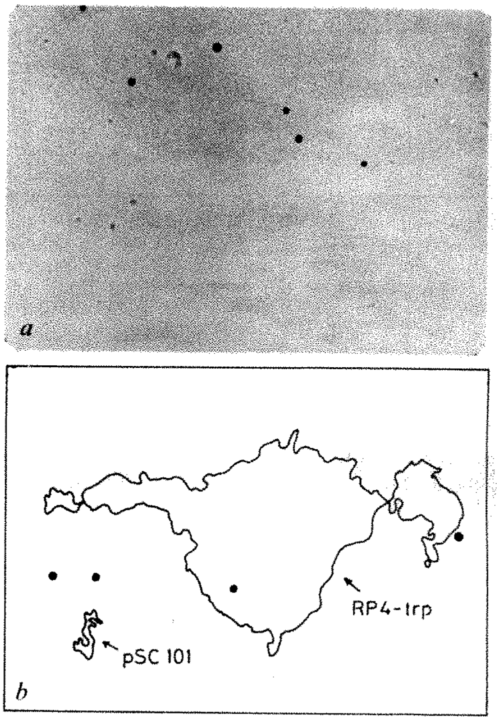

Fig. 1 Electron micrograph of RP4-*trp* DNA obtained from *P. aeruginosa* M12. pSC101 (molecular weight 5.8 × 10^6) was shown as internal standard. RP4-*trp* DNA was calculated to be of 48 × 10^6 molecular weight.

Table 1 shows that the transfer frequency of RP4–*trp* plasmid from *E. coli* cells to *P. aeruginosa* cells was lower by two orders of magnitude than that between *E. coli* $r^-_K m^-_K$ strains, whereas the transfer frequencies of RP4–*trp* from *E. coli* to *P. aeruginosa* for Trp$^+$ and Carb^R markers are almost equal (Table 1), implying that the low frequency is due to the presence of a restriction systems or some other mechanisms in *P. aeruginosa* cells but not to the inactivity of *trp* operon of *E. coli* in *P. aeruginosa*. So far, all the transformants selected for resistance to carbenicillin were also tryptophan independent and vice versa.

Activities of two enzymes belonging to tryptophan operon, anthranilate synthetase (ASase) and the β subunit of tryptophan synthetase (TSase) were measured in crude extracts of cells grown in non-repressed or repressed condition (Table 2). The levels of both enzymes in the *P. aeruginosa* M12 strain (PAO subline) carrying RP4–*trp* plasmid were markedly elevated compared with those in a wild *P. aeruginosa* PAO strain which has chromosomally-located *trp* genes, and showed almost equal enzyme activities in both non-repressed and repressed conditions.

Calhoun *et al.*[9] reported that in wild-type *P. aeruginosa*, the presence of exogenous L-tryptophan during growth in a minimal salts–glucose medium had no influence on the specific activities of ASase, 5-phosphorylribose 1-pyrophosphate phosphoribosyl transferase, N(5′-phosphoribosyl) anthranilate isomerase and indoleglycerol 3-phosphate synthetase in the cells, whereas their real repressibility by

Table 1 Transfer frequency of RP4-*trp* plasmid

Donor	Recipient	Selection	Transfer frequency
E. coli C600 $r^{-}_{\mathrm{k}}m^{-}_{\mathrm{k}}trp^{-}$ (RP4-*trp*)	*E. coli* C600 $r^{-}_{\mathrm{k}}m^{-}_{\mathrm{k}}trp^{-}str^{\mathrm{R}}$	Trp^{+}	1.7×10^{-2}
		Carb$^{\mathrm{R}}$	1.3×10^{-2}
E. coli C600 $r^{-}_{\mathrm{k}}m^{-}_{\mathrm{k}}trp^{-}$ (RP4-*trp*)	*P. aeruginosa* M12 str^{R}	Trp^{+}	1.8×10^{-4}
		Carb$^{\mathrm{R}}$	1.2×10^{-4}

Donors and recipients were middle log-phase cultures grown in nutrient broth supplemented with 0.4% KNO_3 without shaking at 37 °C. 0.5 ml of a donor culture was mixed with 2.5 ml of a recipient culture with 2.0 ml of fresh broth and this mixture was incubated at 37 °C for 2 h without shaking, then an aliquot was plated on the streptomycin (100 µg ml^{-1}) containing Vogel and Bonner's minimal medium in the absence of tryptophan, or in the presence of tryptophan (Trp, 50 µg ml^{-1}) and carbenicillin (Carb, 500 µg ml^{-1}) for transfer to *E. coli*. Appropriately supplemented (as above) glutamate minimal medium[10] was used for transfer to *P. aeruginosa*. The frequency of transfer was calculated as number of transfers per donor cell. Strains used were *E. coli* C600 $r^{-}_{\mathrm{k}}m^{-}_{\mathrm{k}}trp^{-}$ (RP4-*trp*), restriction and modification minus *E. coli* C600 $leu^{-}thr^{-}thi^{-}trp^{-}$ containing RP4-*trp*; *P. aeruginosa* M12 (ref. 10), *trp*.

exogenous tryptophan were revealed only in tryptophan auxotrophic mutants which caused the strict deprivation of intracellular tryptophan in depressed culture condition. In contrast the TSase activities in *P. aeruginosa* were much reduced in the presence of exogenous tryptophan and induced by their substrate indoleglycerophosphate. Our data on PAO (wild type) strain are consistent with their observations, but a striking difference exists in the specific enzyme activities of strain M12, which has received the complete *E. coli* tryptophan operon in the form of RP4–*trp* plasmid DNA. Strain M12 carrying RP4–*trp* showed much higher levels of both ASase and TSase activity, and neither enzyme was repressed by exogenous tryptophan.

The amount of RP4–*trp* DNA in *P. aeruginosa* measured on a CsCl–ethidium bromide density gradient was almost equal to that of RP4 DNA in the same host strain (data not shown), indicating that RP4–*trp* was limited in number as RP4. This confirms our assumption that the expression of the *E. coli* tryptophan operon was out of the control of the *P. aeruginosa* repression system.

The molecular weight of RP4–*trp* in *P. aeruginosa* was 48×10^{6} (Fig. 1), which was the sum of the RP4 DNA (38×10^{6}) and the tryptophan fragment (10.8×10^{6}) derived from $\lambda trpE$–$A_{60\text{-}3}$ DNA. The two bands, one corresponding to 10.8×10^{6} and the other presumably to 38×10^{6} daltons could be seen in agarose gel electrophoresis of *Eco*RI-digested RP4–*trp* DNA obtained from *P. aeruginosa* M12 cells (data not shown), showing that the complete *E. coli* RP4–*trp*[7] was transferred to *P. aeruginosa*. Together with the transfer experiments this shows that the RP4–*trp* DNA has not been changed by deletion or insertion during conjugation.

These results imply that the *trp* repressor protein of *P. aeruginosa* cannot bind to the *trp* operator region of *E. coli* and so the *trp* gene products are expressed constitutively. The *P. aeruginosa* Trp^{-} phenotype has been converted to Trp^{+} by the effective transcription and translation of the *E. coli trp* operon by the *Pseudomonas* system.

Manson and Yanofsky discussed the suggestion that the identical regulation of the *E. coli trp* operon in many of the Entero-bacteriaceae is proof of their common ancestry. The Family Pseudomonadaceae, however, seems to have diverged from the Family Enterobacteriaceae further back in evolution.

Table 2 Specific activities of tryptophan-synthesising enzymes in *P. aeruginosa* PAO1 wild type and M12 carrying RP4-*trp*

Strain	Trp	ASase	TSase β
PAO1	+	1.41	0.38
	−	1.53	2.17
M12 (RP4-trp)	+	6.59	19.0
	−	6.11	21.2

Cells were grown in glutamate minimal medium with or without tryptophan (100 µg ml^{-1}) and harvested in late exponential phase. The preparation of crude cell extracts and the assays for anthranilate synthetase and β subunit of tryptophan synthetase activities have been described[8].

These experiments were carried out using precautions corresponding to the P3 containment level suggested by the US National Institutes of Health. *P. aeruginosa* and *E. coli* are natural hosts of the RP4 plasmid, and the fact that the *E. coli trp* operon is translated effectively in *P. aeruginosa* prove that the translation codes are identical in these two organisms suggests that possibly dangerous 'unknown' proteins would not be produced by this combination.

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Correlation between the X-ray structure of melanostatin and its conformation in solution

THE establishment of correlations between solid-state (for example, X-ray) conformations and conformations in solution (monitored by spectroscopic methods such as nuclear magnetic resonance (NMR)) is essential for the understanding of structure–function relationships in biologically active molecules. The relative functional importance of static and dynamic structures is of particular interest for peptide hormones which reach their site(s) of action on the receptor via a fluid medium. We describe here a comparative study of melanostatin (Pro–Leu–Gly–NH_2) which acts as a melanocyte stimulating hormone release-inhibiting factor[1].

Melanostatin was chosen for study because its X-ray structure is known[2], its minimum energy conformations have been computed[3], and ^{1}H NMR studies in deuterated dimethylsulphoxide[4] and in aqueous solution[5] provide information about the time-averaged values of the dihedral angles in the backbone and side chains. We used ^{13}C NMR spin–lattice relaxation times[6] (T_1) of the proton-bearing carbons of melanostatin, in conjunction with a plausible theoretical model of molecular reorientation, to elicit dynamical information about the conformation in solution. The model for reorientational relaxation is stochastic rotational diffusion, with parameters optimised by a nonlinear least-square procedure[7,8]. From these one can estimate both the overall molecular tumbling rate in solution, and the rates of internal motion about individual bonds. Thus the T_1 data facilitate study of the relative flexibilities of the various molecular fragments.

We first attempted to correlate the ^{13}C NMR T_1 values obtained for melanostatin in aqueous solution with theoretical values calculated for the rigid X-ray structure. This rigid conformation was assumed to tumble about the three principal axes of its moment of inertia tensor with the optimal diffusion rate constants D_i. Even fully anisotropic ($D_1 \neq D_2 \neq D_3$) overall rotation of the rigid X-ray structure cannot account for all the observed T_1 values. This conclusion is unchanged even if the molecule is assumed to take on the unlikely fully extended conformation. Furthermore, the least-squares fit is not improved if the reorientation axes are chosen optimally rather than forced to coincide with the principal axes of the moment of inertia tensor. It was necessary to invoke internal motion for various molecular fragments in order to reproduce the observed T_1 values of melanostatin.

^{13}C T_1 values were measured in aqueous solution (50 mg ml^{-1}) using the inversion-recovery technique[9]. Complete nuclear Overhauser enhancements (NOE) were obtained for all proton-bearing carbons[10]. Table 1 shows ^{13}C T_1 values determined for Pro–Leu–Gly–NH_2 in which the prolyl residue is uncharged. Fig. 1 depicts molecular models of the folded and extended forms of the tripeptide we used in the calculation. The models are viewed along the C and B principal axes of the moment of inertia tensor for the folded and extended forms, respectively. The moments of inertia were calculated from the Cartesian coordinates of all the atoms[11]. The ORTEP thermal-ellipsoid plot programme for crystal structure illustration[12] was used to draw the figures. A nonlinear least-squares procedure[7,8] was used to determine optimal sets of anisotropic rigid body rotational diffusion constants D_i, using experimentally obtained T_1 values and the computed direction cosines of the corresponding C–H vectors. C–H distances were assumed to be either 1.09 Å, or the actual values deduced from the X-ray data; the qualitative conclusions do not depend on the choice of the C–H distances. Table 1 lists the optimal effective correlation times τ_i ($\tau_i = 1/6D_i$) for overall rigid anisotropic molecular reorientation about the principal axes of the moment of inertia tensor, as well as the T_1 values calculated with these correlation times. Results are shown both for the X-ray structure and the fully extended form.

When all proton-bearing carbons are included in the least-squares fitting, the T_1's of the α–carbons are matched reasonably well (within ±40%). T_1 values for the CH_2 groups in the proline ring and for the CH_3 groups of the leucyl residue, however, were only within a factor of 2–3 of the experimental values. It is reasonable to ascribe these discrepancies to flexibility within the pyrrolidine ring of proline[13] (probably puckering motion), and to rapid internal rotation of the CH_3 groups[14]. We repeated the calculations in the absence of these carbons. This led to τ_i values qualitatively the same as in the all-carbon case and which produced T_1 values for the α carbon of the prolyl, and the α, β, and γ carbons of the leucyl residue that were within the experimental uncertainties (±10%) of the measured T_1 values. Of special interest is the discrepancy between observed and calculated T_1 values for the glycyl residue. This is attributed to increased flexibility of this residue with respect to the remainder of the peptide backbone, a feature which is emerging as characteristic of glycyl residues[7].

The overall molecular tumbling of the X-ray conformation is not markedly anisotropic, having effective correlation times of 5.9, 7.5 and 3.0×10^{-11} s for rotation about the principal axes A, B and C of the moment of inertia tensor. Because the overall motion is nearly isotropic, displacement of the principal axes of the rotational diffusion tensor from corresponding axes of the inertia tensor would have little effect on calculated T_1. Calculations were repeated for a fully extended conformation in order to test the sensitivity of the calculated T_1 values to

Table 1 Least-squares fit of rotational correlation times to ^{13}C T_1 values of melanostatin

Carbon atom	Observed T_1 (s)	Calculated T_1 (s)							
		X-ray conformation				Extended conformation			
Pro αCH	1.00	1.35*	0.99*	1.03*	0.96*	0.84*	0.89*	1.03*	0.95*
βCH$_2$	0.88	0.56*	0.48*	0.46	0.42	1.03*	0.92*	0.46	0.43
γCH$_2$	1.23	0.65*	0.44*	0.51	0.42	0.67*	0.69*	0.51	0.39
δCH$_2$	1.00	0.63*	0.48*	0.50	0.39	0.83*	0.77*	0.50	0.44
Leu αCH	0.68	0.77*	0.89*	0.72*	0.75*	0.83*	0.87*	0.72*	0.94*
βCH$_2$	0.43	0.48*	0.46*	0.42*	0.37*	0.62*	0.62*	0.42*	0.48*
γCH	0.90	1.26*	0.93*	1.01*	0.69*	0.83*	0.87*	1.01*	0.94*
δCH$_3$	0.92	0.38*	0.29*	0.31	0.27	0.50*	0.50*	0.31	0.29
δ′CH$_3$	0.92	0.40*	0.30*	0.32	0.27	0.52*	0.50*	0.32	0.30
Gly αCH$_2$	0.63	0.56*	0.48*	0.46*	0.37*	0.60*	0.64*	0.45*	0.46*
Euler angles†									
α		(0)	115.0	(0)	77.0	(0)	−29.0	(0)	55.0
β		(0)	−76.0	(0)	−48.0	(0)	0.0	(0)	−31.0
γ		(0)	51.0	(0)	53.0	(0)	0.0	(0)	36.0
Optimal correlation times‡ (s)	τ_A	5.0×10^{-11}	6.8×10^{-11}	5.9×10^{-11}	1.2×10^{-10}	7.2×10^{-10}	3.9×10^{-11}	5.9×10^{-11}	6.9×10^{-11}
	τ_B	8.0×10^{-11}	5.4×10^{-11}	7.4×10^{-11}	4.9×10^{-11}	2.8×10^{-11}	8.1×10^{-11}	7.5×10^{-11}	7.5×10^{-11}
	τ_C	1.9×10^{-11}	3.8×10^{-11}	3.0×10^{-11}	3.6×10^{-11}	5.9×10^{-12}	7.6×10^{-12}	3.0×10^{-11}	3.0×10^{-11}

*Carbons which were included in least-squares fit.
†Euler angles characterising deviation between rotational diffusion and moment of inertia tensor axes.
‡$\tau_i = 1/6D_i$, where D_i are rotational diffusion constants.
All C–H distances are 1.09 Å.

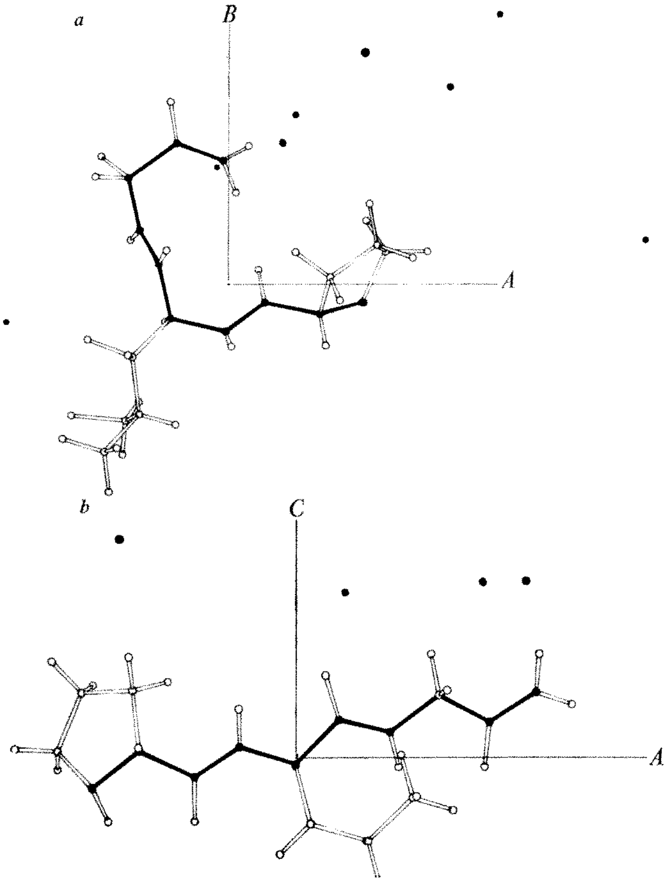

Fig. 1 Conformations of Pro–Leu–Gly–NH_2. Axes are drawn along principal axes of the moment of inertia tensor. *a*, Conformation determined from X-ray crystallography. Model viewed along the *C* axis. Moments of inertia (I) I_A = 1,897.7, I_B = 2,174.4, I_C = 3,661.8 a.m.u.Å^2 (1.00:1.15:1.93). *b*, Extended structure. Model viewed along the *B* axis. I_A = 636.5, I_B = 3,571.4, I_C = 4,037.6 a.m.u.Å^2 (1.00:5.61:6.34).

conformational differences. Even though the overall fitting errors are comparable for the two conformations, the optimal sets of τ_i values are physically less reasonable for the extended form (in some instances spanning three orders of magnitude).

Having established that only internal flexibility can account for the observed T_1 values, we estimated optimal rates of internal rotation about individual C–C bonds. This was done both for the CH_3 groups and for the $C_\alpha^{(Leu)}$–$C_\alpha^{(Gly)}$ pseudo-bond[16] in the backbone. The values for the effective correlation times τ_A, τ_B, τ_C describing overall molecular reorientation of the X-ray conformation were the optimal values obtained fitting the backbone carbons only. Using an ellipsoid of rotation for the overall molecular shape, the effective correlation times we calculate for the CH_3 rotation ($\tau_{int} = 5 \times 10^{-12}$ s) and for rotation about the pseudo-bond ($\tau_{int} = 5.9 \times 10^{-11}$ s) are physically reasonable, further confirming the postulate of internal mobility. No attempt was made to estimate the rate and amplitude of puckering in the proline ring.

Correlating the X-ray structure with ^{13}C NMR relaxation data has shown that in solution Pro–Leu–Gly–NH_2 is not rigid on the NMR time scale. The T_1 data seem to be good qualitative monitors of internal mobility in linear peptides, and when interpreted on the basis of simple theoretical models of rotational reorientation, can identify and locate flexibility within the molecule. The overall molecular shape of peptides the size of melanostatin (and up to molecular weight ~1,000) has minor influence on the calculated rates of internal rotation about individual C–C bonds[15]. Thus side chain–side chain interactions and their influence on the mobility of adjacent residues in peptides and peptide hormones can be profitably studied using ^{13}C NMR relaxation measurements in solution, without the need for precise information on the overall molecular shape. Such studies, coupled with experiments on the influence of solvents on T_1 (which probe changes in mobilities or structure due to the changing environment), with rotamer population data derived from spin–spin coupling measurements, with proton–proton relaxation results, and with predictions from conformational calculations, can provide consistent dynamical information about peptides in solution and may enhance our understanding of structure–function or conformation–function relationships in peptide hormones.

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Errata

In the article 'Mantle helium in the Red Sea brines' by J. E. Lupton, R. F. Weiss and H. Craig, *Nature* **266**, 244–246, the labels 714–G8 and Discovery in column 1 of Table 1 should be transposed.

In the article 'Direct demonstration of β-globin mRNA in homozygous Ferrara β_0-thalassaemia patients, the author sequence should have read S. Ottolenghi, P. Comi, B. Giglioni, R. Williamson, C. Vullo, L. del Senno and F. Conconi. Figure 3 description line 2 should read Nuclear (*a*) and cytoplasmic (*b*) . . .

Corrigendum

In the article 'Punishment learning and self-mutilation in Lesch–Nyhan disease, *Nature* **265**, 461–463, sentence 1 of paragraph 2 should read 'Lesch–Nyhan disease is an X-linked recessive disease . . . '.

matters arising

The 17-d periodicity of Cygnus X-3

ARIEL V observations have indicated[1] a possible 16.75-d periodicity in the flux of Cygnus X-3. I report here that the primary data (from ref. 1) have been digitised and power-spectrum analysed; the results are shown in Fig. 1. (Analysis details available from the author.) The power spectrum shows a broad continuum of fluctuations, with an r.m.s. variation ~ 40% of the average flux for periods $\gtrsim$ 3 d and a power law spectrum, $P(f) \propto f^{-1.5}$. This is not the spectrum expected from a shot-noise source[2].

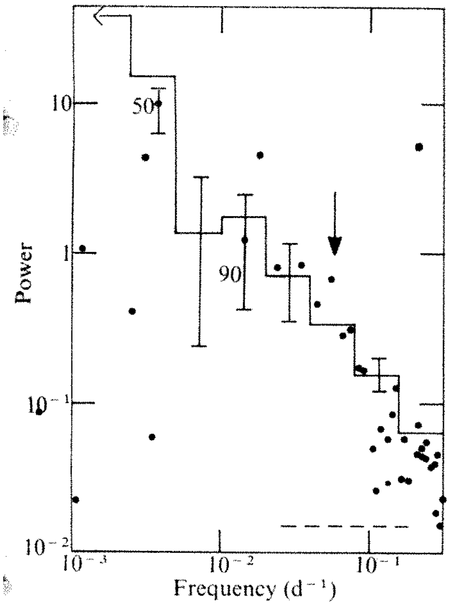

Fig. 1 Relative power spectrum of the flux of Cygnus X–3. Circular points are spectrum using FFT method (typical 50% and 90% confidence intervals are shown). The histogram gives spectrum using nested variance technique (90% confidence intervals shown). Dashed horizontal line represents noise level.

The arrow in Fig. 1 indicates a small peak in the power spectrum at the 16.75-d periodicity discussed in ref. 1. The significance of the peak is estimated from the excess power above the background fluctuation level (rather than above the Poisson noise level)[2]. There is about 50% likelihood that there exists a 16.75-d periodicity. This periodicity, if real, accounts for ~10% of the fluctuations and has an r.m.s. amplitude ~4% of the total flux.

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HOLT REPLIES—Owens implies that the ~17-d periodicity reported in ref. 1 may be artefactual, in view of the broad continuum of fluctuations evident in the raw data. We have no quarrel with such a qualification; indeed, ref. 1 explicitly pointed out that the high degree of source variability, variable gaps in coverage, and no explicit correction for the known 4.8-h source modulation could be expected to result in artefactual frequency components in the data analysis.

The ~17-d effect was not, therefore, reported solely on the basis of it being the most significant (statistically) period in the search we performed on the Ariel V All-Sky Monitor data. We felt that the apparent presence of the same effect in the Ariel V Sky Survey Experimental data, which were corrected for 4.8-h variations and which were obtained during extended gaps in the All-Sky Monitor exposure (so that the two sets of data were completely independent), was sufficient justification for alerting the scientific community to the possibility of its reality. We regret that the quality of our data cannot support a stronger statistical argument for the presence (or absence) of a persistent ~17-d modulation of the variable Cyg X-3 intensity.

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No new limit on size distribution of γ-ray bursts

Carter *et al.*[1] derived an upper limit to the (balloon) intensity of small γ-ray bursts at ≈ 100 below the extrapolated known size spectrum, and concluded that γ-ray bursts are of galactic origin. But we claim, for the following reasons, that their results are entirely consistent with the −1.5 index power-law extrapolation. There is, therefore, no conclusion regarding the nature or the origin of γ-ray bursts to be drawn from their measurements.

(1) Their selection criterion for candidate events was that of three successive 0.6-s increases in the γ-ray count rate. A study of all known bursts (data kindly supplied by R. W. Klebesadel) shows that this requirement ignores 75% of events due to their widely varying temporal nature.

(2) The total energy sought in 1.8-s does not represent the entire energy that would be found, for the same reason. The missing energy due to fluctuations is incorporated by reassigning the event size by a factor of ≈ 2.

(3) Observed γ-ray burst size spectra have been published[2,3] with the measured flux of each known event multiplied by 1.4 to 2, to include the emission below the satellite energy threshold, knowing the typical spectra[4–6]. Comparison data must normalise equivalently.

(4) The authors knowingly employed a 1-s.d. upper limit[1]. Small-number statistics demand the usual confidence limit of 95%, raising their upper limit by three.

(5) The observed fraction of the sky was apparently taken to be 0.5, ignoring atmospheric absorption at large zenith angles which would give an unobscured sky fraction of 0.25–0.3. This point is incorporated within the following considerations.

(6) The response of a horizontal, flat detector must distort the measurement of the burst size spectrum, even from an isotropic source distribution. For a totally absorbing detector it can be shown[7] that the average burst size $\overline{S}$ that contributes to the smallest events of apparent size $Z = S \cos\theta$, given an intrinsic spectrum $N(S)\,\mathrm{d}S = kS^{-\gamma}$, is

$$\overline{S} = Z\,[(\gamma-1)/(\gamma-2)]$$

$$\times\ [(1-\cos^{(\gamma-2)}\theta_0)/(1-\cos^{(\gamma-1)}\theta_0)], \quad (2)$$

with θ_0 the maximum equivalent unobscured zenith angle θ. Using their published detector characteristics[1], we find a required vertical shift of 2.2 and a horizontal shift of 1.3 on the size spectrum, including point 5.

The total combined effect is that the upper limit rederived from the data of Carter *et al.*[1] is raised up by two orders of magnitude, entirely consistent with the −1.5 index power-law extrapolation of the known size spectrum (Fig. 1). In fact, it is exactly an analogous threshold effect of lack of observation which depresses the Vela size spectrum low end by up to three orders of magnitude.

Even if (on the basis of no single-event observation) an upper limit below the −1.5 index extrapolation were found, this alone would not prove either the size spectrum model or the origin hypothesis suggested[1]. Such a result would imply only that the source distribution is not infinite in extent, assuming that the average absolute magnitude of emitters is independent of distance.

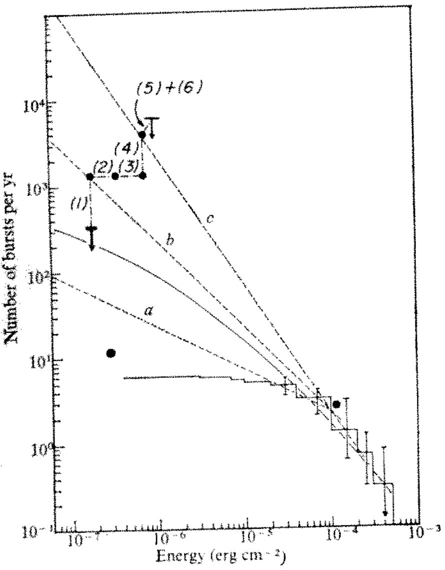

Fig. 1. The γ-ray burst results of *Carter et al.*[1], adjusted by six factors, numbered accordingly. Compare with Fig. 1 of ref. 1.

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CARTER ET AL. REPLY—Cline and Schmidt[1] have presented a series of subjective arguments in an effort to show that our earlier reported upper limit to the rate of γ-ray bursts[2] can be made to conform with a hypothetical number–size spectrum with an index of −3/2. We believe that they have become too attached to this hypothesis. In fact, the data from the Vela satellite is equally consistent with an index of −2, although this does not have the same cosmological attraction. If the power-law index were −3/2 then a detector with an energy of 10^{-7} erg should record a burst approximately every 2 h or so. No such evidence exists.

Our criterion for the recognition of γ-ray bursts was that the counting rate in each of three consecutive, and independent, measurements should exceed the background by 3σ. Unless one has a coincidence in a second, remote, sensor it would seem imprudent to claim excesses in a single measurement interval to be a genuine γ-ray burst. There is evidence of some poorly understood events in which anomalous increases arise in a single resolution element as well as effects which lead to the occasional saturation of a complete telemetry frame[3,4]. Since the basic resolution time in our particular experiment was 0.6 s, an intrinsic limit of 1.8 s is set in our burst search. Although our main data analysis was confined to the above selection criterion, we did in fact search for possible shorter events. In this case, however, we required a coincidence in the counting rate from at least two of the differential energy channels. No evidence for γ-ray bursts was detected using this criterion either.

We had not discussed the rationale of our selection criterion nor the details of our analysis methods, in our original brief contribution since they are fairly standard procedures.

In the following sections we comment on the numbered points raised by Cline *et al.*

(1) From the existing published data[5,6] it may be seen that the typical size for a γ-ray burst is 3–5 s and the first peak lasts ~2 s. The temporal variations seem to be of the same magnitude as the basic time resolution of the particular experiment. Our selection criterion, therefore, will be met by a substantial majority of these events. We will, admittedly, lose those events having a duration ⩽1 s but we estimate this loss to be about 20% of the total.

(2) The limit shown in our figure corresponds to the selection criterion outlined above. We could have set a lower energy limit had we considered only longer bursts. But, since the typical length of γ-ray bursts is longer than our measurement interval and since the average intensity represents the first moment of the generating function, the upper limit deduced on this simple average basis is an adequate representation of the burst energy.

(3) The correction factor suggested here seems rather naive. First, there is no experimental evidence that the spectra of all bursts extend below 100 keV. From a comparison of the OGO and Vela results we estimate that only 60% of the events would meet this criterion[7]. Second, if the burst spectrum did, in each case, extend below 100 keV, the correction factor for the missing energy would be a function of the particular experiment. The use of a constant factor as suggested by Cline and Schmidt is absurd. The correction factor in our case was estimated to be 1.3.

(4) This point was adequately discussed in our text which we had expected to have been regarded as an integral part of our presentation.

(5) When atmospheric effects, including multiple-Compton scattering had been taken into account we estimated that the effective field of view of our detector was ~1.5π sr.

(6) The comments raised in this section have been discussed in the past in the context of cosmic-ray physics. Deductions from the mathematical treatment suggested by them is unwarranted in the absence of any established power-law index. The expression suggested by Cline and Schmidt is clearly erroneous since the value $\overline{S}/Z$ can be seen to be independent of the zenith angle for $\gamma = 3/2$. The expected number of bursts with a detector floating in the atmosphere can be written as

$$N_B = \Sigma_i\, w_i\, N(E_i)$$

where w_i is the solid angle and E_i the average threshold energy in the ith element. The expression can be solved to the required degree of accuracy with a knowledge of $N(E)$, the integral size spectrum of γ-ray bursts which is as yet unknown. Alternatively, the correction may be evaluated approximately, in a more practical way, independent of the size spectrum. For a given detector the energy deposited by a γ-ray burst must lie between E and $3E$ for zenith angles ranging between $\theta = 0$ and $\theta = 75°$. This is due to a decrease in the effective area by a cosine factor. The detection efficiency at large zenith angles, however, increases due to increased path length in Na I by sec θ. Therefore the detector can be regarded as being less sensitive by a factor of about two. Such a consideration will make our earlier results an absolute upper limit for bursts with energy greater than 5×10^{-7} erg.

We conclude that the second-order refinements discussed above can, at most, contribute a factor of about two, both in burst energy and in burst rate. Cline and Schmidt are entitled to make their own assessment of our results but we maintain that these corrections do not materially change our conclusions and that, at this stage, we see no compelling reason to reconcile our results with their hypothesis.

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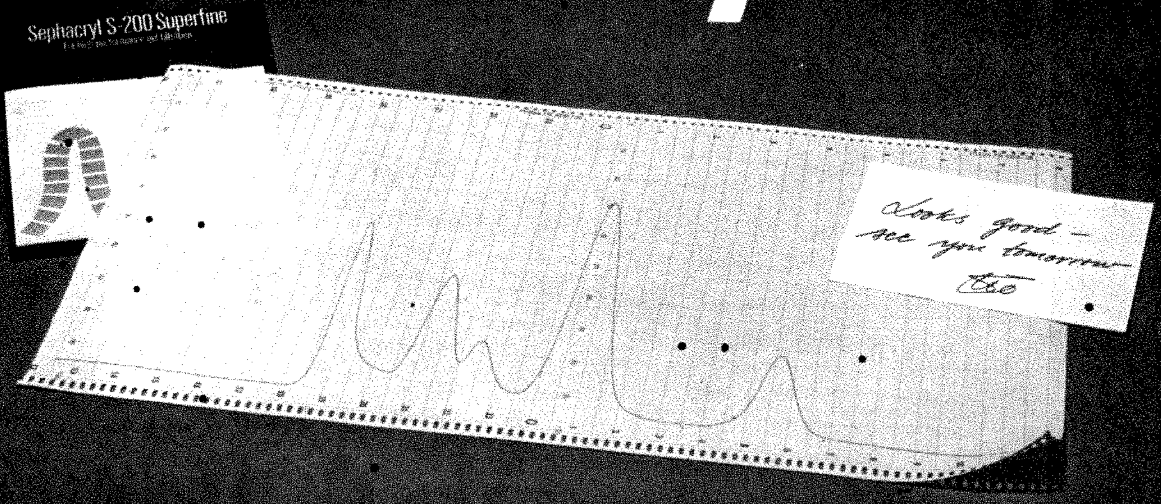

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reviews

Nutrition and the developing brain

John Dobbing

Malnutrition and Intellectual Development. Edited by J. D. Lloyd-Still. Pp. 194. (MTP: Lancaster, 1976.) £8.95.

A SMALL epidemic of recent books on or around this topic imposes a special need to justify the addition of any new one to the bookshelves. Dr Lloyd-Still's is an edited volume of six reviews or essays, dealing in turn with: effects of undernutrition on the growth of the physical brain and on "mental competence"; techniques of psychological testing in this context; some studies of the subsequent physical and intellectual development of children inevitably malnourished in the course of certain clinical conditions in affluent society compared with eleven major studies from the underprivileged world; and finally the "social ecology" of malnutrition in childhood. It is nicely produced, and there is certainly enough excellence to justify its place alongside other recent books (for review, see *Nature*, **262**, 521, 1976).

After a very thoughtful introductory chapter by the editor in which he gives a very balanced description of the whole subject, there is a disappointing second chapter by Peter Chase on the physical effects of undernutrition on the growing brain. I think my dissatisfaction with it largely derives from Chase's total lack of humility before the amazing complexity of the developing brain. Where the rest of us use curves of brain weight, cell number, myelin lipid and so forth as crude indicators of the timing of developmental events so as to identify hypothesised periods of enhanced vulnerability. Chase seems to think of such parameters as of specific significance in themselves. They are, of course, merely the things we can conveniently measure. Some of his graphs of their accumulation in whole brain are misleadingly wrong in places. And who is interested in whole brain quantities anyway, except in the limited sense mentioned above?

The third chapter by epidemiologists Zena Stein and Mervyn Susser draws heavily on their fascinating study of the effects on fetal growth and 19-yr-old achievement following the Dutch wartime famine during the winter of 1944–45. This is a beautifully written, distilled account of their findings, cleverly described in the setting of many other epidemiological studies, including some in which there is experimental nutritional intervention. The book deserves to be bought for this chapter alone.

The Hurwitz chapter on psychological testing in studies of malnutrition is very useful in a different way. Assuming his descriptions of the test methodology are reliable (and I cannot judge that), I was most impressed with his account of the limitations of such tests in the present context. The problems of cross-cultural validity together with the difficulties of drawing "growth-curves" against age in longitudinal studies, seem even more formidable than one had imagined, to say nothing of the hazards in using infant tests as predictors of long term performance.

Lloyd-Still returns in Chapter 6 to discuss "Clinical Studies", by which he means direct studies on children. His tabulated condensation of 13 major studies includes 11 of socioeconomic or cultural malnutrition and two in which malnutrition was secondary to clinical conditions in our own privileged communities. The breadth of his discussion is impressive and partly derives from his conspicuously profound experience as a children's doctor. One day, however, we shall have to abandon discussion of some of the earliest field studies since it is now too easy and somewhat redundant to criticise them. Their place is in historical introductions to theses.

The last chapter on the social ecology of childhood malnutrition unfortunately returns to the rather lower standard of the second. It may well be true that the "Soviet Union, which was able to purchase . . . grain with devalued dollars at prices subsidised by the United States Government, realised further profit, both economic and political, by selling, lending and granting of American grain to other countries"; but such statements are below the generally very high standards of objectivity in the remainder of this book. □

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Electrons in liquid ammonia

Electrons in Liquid Ammonia. By J. C. Thompson. Pp. xii+297. (Clarendon: Oxford; Oxford University: London, 1976.) £14.50.

THIS book is a gold mine of information for anyone working in any area of electrons in fluids. It is written by a person who has contributed to all of the many areas represented in this monograph. Professor Thompson has not only collected all of the major experimental and theoretical work on the subject but has presented it in a very critical but very fair manner. After reading this book everyone should have a better feeling for our present state of understanding as well as the remaining problems.

The major topics include "metal–ammonia solutions as liquid metals", "metal–ammonia solutions as electrolytes", "the metal–nonmetal transition", "liquid–liquid phase separations", "solutions of alkaline earth and rare Earth metals", "solid metal–ammonia compounds", and "solutions in other solvents". Each chapter contains material which will prove useful to even the most well informed reader on the present status of each topic. The presentations are remarkably up to date and inclusive.

The strongest single point of this book is its emphasis on those areas in which our understanding of experimental data is very incomplete. In this regard one might mention spin pairing and the "metal–nonmetal" or conductivity transition. In these cases the author has made a special effort to quote all of the essential experimental facts as well as to make the stongest possible case for all of the alternative theoretical models (pointing out in addition that all fail on some other points).

This book is so vital that any researcher in any related field would be negligent if this book were not actively used as part of his routine research. **Neil R. Kestner**

Neil R. Kestner is Professor of Chemistry at Louisiana State University, Baton Rouge, Louisiana.

Human immunology

Immunology of Human Reproduction. Edited by J. S. Scott and W. R. Jones. Pp. xviii+476. (Academic: London; Grune and Stratton: New York, 1976.) £15.

THIS is a fascinating book for all those who have an interest in immunology and development. The book begins with a glossary and a primer of immunology that has freed later chapters from the need to define and explain. This introductory section is oversimplified in places and occasionally patronising, but these faults are outweighed by the good it does in summarising and ordering a field overgrown with observations and special terminology.

Although no formal break is made, I would divide the remaining chapters into two sections: chapters 2–6 dealing with basic aspects of immunology in development, and drawing on both clinical material and animal, model, experiments; and chapters 7–13, dealing largely with clinical aspects of human immunology and development. The chapters are of varied length, quality and distance from the stated topic of the book, but they all elaborate their topics in considerable detail, and it is this detail that is the book's strength.

The authors themselves are often moved by the complexities and uncertainties of the topics, to use phrases like "the tip of the iceberg of confusion" or to make statements such as "existing knowledge concerning the role of immunological factors in gestosis is fragmentary . . ." or "the picture . . . as in so many areas of reproductive immunology is confused . . ." This confusion is, however, the result of contradictory or insufficient clinical and experimental observations, and by detailing these the authors have done a great service, allowing others to speculate on, and experiment over, the data presented here.

The fields of immunology and reproduction are connected at many points. Chapter 2, by M. H. Johnson, makes a connection between the two by discussing the stages of early mammalian development (exemplified by the mouse embryo) and some ways in which immunological techniques may be used to characterise these stages. In this chapter the problem is raised of the maternal immune response to antigens of the embryo, either to alloantigens, or to stage-specific antigens. This problem, still unsolved, is reiterated in later chapters, notably that by Billington on the immunology of trophoblast, and in a chapter by Medenhall, which considers all aspects of maternal–foetal immunology, transfers of maternal immunoglobulins and cells, and the problem of the foetal allograft. A further comment on the problem is made by Gusdon, in a chapter on the effects of pregnancy on all maternal immune responses. The first section closes with a chapter by W. Jones on the phylogeny and ontogeny of the immune response. This chapter considers development of foetal immune responses in its own right. It is perhaps too broad and contains a few notable factual errors (the mouse is assigned a 30-d, not a 20-d gestation period), but the section on the maturation of the immune responses of the human foetus is thorough, including the immunological aspects of breast feeding, and quite interesting.

In the second section we are given extended treatments of particular topics. Some, such as that of chapter 7 on the consequences of maternal infection for the foetus, cut across the divisions set up in the earlier chapters. Others, such as a chapter on the effect of pregnancy on immunological diseases of the mother, analyse a limited topic within the larger one already set. Maternal–foetal immunological interactions are prominent in Rh disease, and this topic receives full treatment in a chapter which emphasises clinical aspects of the disease, although failing to discuss the molecular aspects of Rh antigens and of the transfer of Rh antibodies across the placenta. Gestosis (eclampsia/preclampsia), a condition involving the immune response in some obscure manner is also given a chapter of its own. If we are not convinced at the end of this chapter that the condition is due to immune responses, neither are we put off further exploration of the problem. Similarly, the role of the immune system and of cell surface antigens is unclear in cases of trophoblastic neoplasms. Again, the question cannot be answered but it is well set out and elaborated. Other chapters cover non-gestational reproductive tract neoplasms, and immunological aspects of infertility as well as the prospects for immunology in the control of fertility. All of the chapters carry long lists of references: the average is about 130; and one chapter, that by Edwards on immunology and fertility control contains 390.

The immunology of reproduction is a vast subject and, as will be seen from the individual subjects mentioned, may be taken in many senses. It would be too much to expect a consistent exposition of the subject; indeed, such an exposition would certainly be highly biased, if not false. The opposite approach taken here, if it occasionally daunts the authors, stimulates their readers.

Michael Edidin

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Volcanic picture book

Volcanoes and Impact Craters on the Moon and Mars. Pp. 463. By P. Leonardi. (Elsevier Scientific: Amsterdam and NewYork, 1976.) Dfl.166; $59.75.

AT a first glance this book is very impressive; it is beautifully presented and contains large numbers of high quality pictures, both in colour and in black-and-white. For a subject which relies much on interpretation of photographs taken of other planets, this is highly commendable. The author has not only reproduced the standard NASA and Soviet pictures taken from spacecraft but has also included many extremely interesting pictures of geological phenomena on Earth.

Although the book was originally written in Italian it has generally speaking been well translated into English and reads easily. The author has obviously read widely around this subject and there can be few planetary scientists who will be disappointed at not finding their name in the index.

In view of these comments the reviewer would have liked to have recommended this book. But although pictorially the book is very much up-to-date (even containing Viking pictures), many of the ideas expressed in the text are reiterations of ideas current among some scientists in this field more than ten years ago, before spacecraft exploration of the Moon and planets. Much of the book is devoted to the old argument of impact as against volcanic craters on the Moon, and although the author presents the 'impactists' case fairly, his conclusions lie well within the 'volcanic' camp and would not be accepted by most planetary workers who have studied the evidence obtained during the past ten years.

Sadly, although recommended for its presentation and picture quality, this book will be of little interest to scientists in the field and can only be recommended to students for the historical interest of the text.

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Membrane enzymes

The Enzymes of Biological Membranes. Vol. 1: Physical and Chemical Techniques. Pp. xiii+257. £10.50; $20. Vol. 2: Biosynthesis of Cell Components. Pp. xvii+654. £28; $55. Vol. 3: Membrane Transport. Pp. xv+459. £21; $39. Vol. 4: Electron Transport Systems and Receptors. Pp. xv+431. £20; $37. Edited by A. Martonosi. (Wiley: London, 1976.) (US edition published by Plenum: New York, at slightly different prices.)

THESE volumes form the first four of a series which sets out to collect together and evaluate information on membrane enzymes. Most of the information is concerned with the relationship of enzymes to their membrane, and with their involvement in membrane phenomena. Very detailed information on the structure of each enzyme is usually lacking, since the purification and structural analyses of most membrane enzymes have not advanced as far as for their soluble counterparts.

Some membrane enzymes are dealt with singly, others appear as part of enzyme systems. It is clear from the volume titles that the word 'enzyme' has been interpreted very loosely. Thus, in addition to proteins with clear enzymic activity, the coverage includes binding proteins of various persuasions, transport components without enzymic activity, and light-trapping systems; in short, membrane proteins with clearly documented biological function. This is a reasonable extension in the context of an attempt to provide an insight as to how membranes function. Very few important 'enzymes' have been omitted.

Each volume is a multi-author effort; the cast-list is impressive and most authors do their subject (and themselves) justice. The coverage given to different 'enzymes' varies; in some cases this is an overall view of the 'enzyme' and its relationship to the membrane, whereas in other chapters the emphasis is more firmly on recent developments. On the whole, most contributors reflect the state of knowledge in each field but occasionally reveal a personal bias which may limit the usefulness of their chapter.

In the first volume the uses of various techniques are discussed in establishing the general fluidity of the membrane lipid layer, in demonstrating a collar of lipid around the inserted portions of some proteins, and in interpreting the response of some enzymatic activities to temperature change; all of these discussions use suitable examples. Much of the same work is, however, discussed again in other volumes in relation to the properties of the individual enzymes used as the examples in volume 1. This excessive overlap could have been avoided, as could the length of those contributions taking over 30 pages to discuss a single 'enzyme'.

Volume 4 is a very artificial assembly; the few chapters on receptors, with the exception of that on rhodopsin, have little in common with those on electron transport systems. The receptor chapters would have been better separated off into a further small volume or would have associated better with the transport components. Such considerations are clearly of importance to purchasers of individual volumes but of less importance when considering purchase of the series for a library.

To have such a convenient and comprehensive source of material on membrane enzymes will be a considerable asset in both teaching and research. The series is clearly one for library purchase since it is too comprehensive and expensive for individual subscription. Each volume deals with a coherent group of topics (with the artificial addition of the receptor section to volume 4); although expensive, they are therefore more attractive for single volume purchase than many serial publications.

Roger Coleman

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Disease resistance in plants

Defence Mechanisms of Plants. By B. J. Deverall. Pp. 110. (Cambridge University: Cambridge, London and New York, 1977.) £5.50.

THROUGHOUT their lives plants are exposed to infection by many parasitic microorganisms. Yet infection often fails and the plants remain healthy. Professor Deverall's excellent book is concerned with the processes by which plants perceive the parasite, and occasionally permit but usually discourage its further progress.

Professor Deverall does not attempt an extensive account of the subject but rather chooses to present a selective personal view of recent research pertaining to the processes of resistance to disease. Emphasis is placed on events which occur after a plant has been infected and covers cytological changes, the production of antimicrobial compounds (phytoalexins), induced resistance and the mediation of host–parasite specificity. Each chapter contains a summary of the facts, which is well supported by reference to original and review papers, an assessment of their significance or otherwise, an indication of the problems remaining, and in some cases how they may be solved. Few significant errors or omissions have been noticed although the promise to expand the concept of infection type to all host–parasite interactions is rarely fulfilled.

This book is extremely timely in that it appears alongside several other publications, which although containing more detailed information often fail to analyse it critically. In this respect, Deverall, rather than merely providing a list of phytoalexins, presents a welcome evaluation of the evidence for their rôle in resistance and the mechanisms of their induction. The close association between cell death and phytoalexin synthesis rightly receives much emphasis, but his conclusion that phytoalexins are synthesised in living cells may be somewhat premature.

Professor Deverall states that he envisages his readers to be research workers and university lecturers, and their advanced students. The book can be recommended as essential reading for such people. It will convey the progress achieved by and the challenges remaining for physiological plant pathologists.

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Atmospheric chemistry

Atmospheric Chemistry. By J. Heicklen. Pp. xiv+406. (Academic: New York and London, 1976.) $38; £27.

In view of the rapid expansion in knowledge of the chemistry of the Earth's atmosphere in recent years, publication of a new volume on this topic is timely. *Atmospheric Chemistry* by Julian Heicklen, however, does not in any way achieve the standard of information collection, dissemination and presentation in the two notable publications on this topic in the early 1960s: Leighton's *Photochemistry of Air Pollution* and Junge's *Air Chemistry and Radioactivity*.

The reason for this failure mainly arises from the authors attempt to cover too much ground; indeed some of the topics discussed—such as the kinetics and mechanism of hydrocarbon combustion, the detailed effects of urban pollutants on plants and animals, and pollution control methods—are inappropriate to the field encompassed by the title. On the other hand there is inadequate coverage of the basic large-scale and local meteorological factors determining the transport mechanisms that are so important in defining the reactor in which the chemical reactions take place.

In addition, the overall emphasis is very much towards photochemistry. Although it is true that the absorption of solar radiation is the primary driving force for chemical reaction in the atmosphere, the chemical interactions of trace gases with precipitation elements and the wash-out and rain out processes have an important part to play in the overall atmospheric cycles, and these are hardly discussed at all. This lack of balance detracts from the overall usefulness of the work in presenting an overview of the science of atmospheric chemistry.

The author must be credited, however, with the collection of a large amount of photochemical and kinetic information on the chemical reactions which may occur in the atmosphere. In the chapters on chemistry of the upper atmosphere and on photochemical smog, simple model calculations are developed to demonstrate how the trace gas composition in each particular atmospheric region can be simulated using the chemical kinetic information. This is basically a useful presentation, but unfortunately the arguments tend to become too complex and difficult to follow. Also, the uncertainties in applying the results to real atmospheric situations are not emphasised. For example, in the discussion of a model photochemical smog system, the reader is left with the impression that ozone concentration in sunlit exhaust-polluted atmospheres are governed solely by the photochemical reactions. The important and often dominant role of meteorology, in determining ozone concentrations is not mentioned.

The lack of emphasis on precipitation chemistry is very apparent in the chapter dealing with SO_2 chemistry. A comprehensive 10-page summary is given of laboratory data on SO_2 photochemistry, a process which is widely accepted as being of negligible importance in the atmospheric chemistry of SO_2. The droplet-phase oxidation of SO_2 is, on the other hand, dealt with in a cursory fashion with no reference to some of the early classic work carried out on this important system.

In addition to the above mentioned chapters, there is a concise description of the chemistry of the ionosphere, a long chapter on atmospheric pollutants, which contains very little atmospheric chemistry and a lot of useless information on concentrations in US cities and deleterious effects, together with a short but not very illuminating chapter on aerosol chemistry. A chapter entitled reactions of O_3 and singlet O_2 also contains a detailed discussion of NO_2 photolysis.

There are some misconceptions and inaccuracies which are unsatisfactory for a book of this kind. For example, it is stated that "The troposphere is a relatively quiescent region (of the atmosphere) where relatively little chemistry occurs . . . The global atmosphere chemical cycles can be summarised as mainly oxidation occurring in the stratosphere . . .". This statement is manifestly untrue. In fact, nearly all of the gaseous material emitted into the atmosphere from the Earth's surface fails to penetrate the tropopause. It is oxidised or otherwise scavenged in the troposphere where 80% of the atmospheric mass resides.

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Ion cyclotron resonance spectrometry

Ion Cyclotron Resonance Spectrometry. By T. A. Lehman and M. M. Bursey. Pp. xi+230. (Wiley-Interscience: New York and London, 1976.) £15.95; $27.30.

Ion cyclotron resonance (ICR) spectrometry has been widely used during the past 10 years for the study of the chemistry of ions in the gas phase. Although it has found many applications in the field of chemical physics, it has been used increasingly in studies of the structure and reactivity of organic ions and in the elucidation of reaction mechanisms which parallel those observed in solution. It is on the latter group of applications that this volume concentrates, and by far the longest chapter in the book is devoted to a discussion of such topics as gas-phase acids and bases, nucleophilic and electrophilic substitution reactions, and structures of ions. It is written from the viewpoint of a physical/organic chemist and a number of interesting comparisons are drawn with results obtained from studies of solution kinetics.

This illustrates one of the major problems in writing a book on a particular experimental technique: the subject of gas-phase ion chemistry is much bigger than ICR spectrometry and by confining themselves largely to examples based on ICR studies, it can be argued that the authors present an incomplete and selective view of the field. For example, results obtained from high pressure mass spectrometry or by flowing afterglow and flowing after-drift methods have proved of considerable interest; and although comparisons are drawn between these results and those obtained by ICR spectrometry, discussionof them is necessarily brief.

The first two chapters of the book provide a very readable introduction to the technique in which the reader is encouraged to explore the possibilities of the technique without worrying too much about the finer points of its theory of operation. The chapter on reaction mechanisms is followed by a discussion of theoretical aspects of power absorption, linewidths and extraction of rate data together with a brief review of current theories of ion molecule reactions at near thermal energies. The final chapter briefly describes various applications of ICR spectrometry in the field of chemical physics. The book concludes with an extensive bibliography and two appendices.

The book provides a useful introduction to ICR spectrometry and to physical/organic aspects of gas-phase ion chemistry but the chemical physicist will find little which is not adequately covered in review articles. The problem with any book on a particular technique is that it is necessarily limited in its scope; and although the reader will know quite a lot about ICR spectrometry, if he gets through this book, he will not be in a position to assess its advantages and disadvantages relative to other techniques used in the study of gas-phase ions.

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obituary

Sir Gerard Thornton

MICROBIOLOGISTS, especially those who had the good fortune to work with him, will have heard with sadness of the death of Sir Gerard Thornton on 6 February at his home in St. Albans at the age of 85. He is survived by his widow, Gerda, who is Danish, and by his son Peter who is Keeper of Woodwork and Furniture at the Victoria and Albert Museum.

He will be remembered for his many original and important studies on soil microbes, made in the course of a long career spent almost wholly at Rothamsted Experimental Station, Harpenden. He was educated at Radley and at New College Oxford and joined Rothamsted shortly after the end of the Great War where he had served as a pilot in the Royal Flying Corps.

In 1919, soil microbiology was a very rudimentary science, and Thornton's early work was much concerned with the devising of techniques for isolating, identifying and counting various kinds of soil bacteria and in promoting parallel studies by others on soil fungi, protozoa and other groups. With his friend the late R. A. Fisher he pioneered the application of statistics to the problems of enumerating bacteria in soil. At this time he was also in contact with the great European soil microbiologists—Beijerinck, Winogradsky, Kluyver and later with Waksman. He had a special interest in the microbial breakdown of aromatic disinfectants in soil, and made a collection of bacteria able to utilise cresols, phenols and naphthalene as sole carbon sources and elucidated some aspects of their physiology. This work was later to become specially relevant to the discovery, which he shared with others, of the selective hormone herbicides (chlorinated phenoxyacids), first published in *Nature* (**155**, 498; 1945).

His greatest influence, however, was through his distinguished research on biological nitrogen fixation, especially by root-nodulated leguminous plants. His detailed investigation of their microbiology, structure and physiology laid the foundation for nearly all later work and led to practical applications in UK agriculture. His work established the 'bacteroid' as the nitrogen fixing site in the nodule and he described in detail the features of ineffective, non-fixing nodules. With his Rothamsted colleagues he was the first to use serological methods for identifying rhizobia and was associated with early work on bacteriophage and with studies on ecology of rhizobia. Thornton was also the first to investigate effects of competition between strains in the soil, a matter later shown to be important in the development of efficient 'inoculants' for leguminous crops. Since his retirement in 1957 he continued to show the greatest interest in nitrogen fixation and in the striking advances made world-wide in its study during the last decade, stimulated by the International Biological Programme and the energy crisis.

Thornton had wide interests in science and in the arts. He was especially interested in archaeology and in palaeontology and had the distinction of discovering a new species of plesiosaur (*Rhomaleosaurus thorntonii*) which can be seen at the British Museum, Natural History.

Thornton became head of the Department of Soil Microbiology in 1940. He was elected to the Fellowship of the Royal Society in 1941, of which he was Foreign Secretary from 1955–60 and a Vice-President in 1959–60. He was actively involved in the organisation of the Society's Tercentenary celebrations and was knighted in 1960. He served on the Council of the Royal Society and on many bodies concerned with microbiology and with agriculture.

Thornton was a quiet, modest man, invariably considerate and courteous with a charm of manner rarely seen today. His colleagues remember him with respect and affection.

P. S. Nutman

D. E. Wheeler

DR D. E. WHEELER who died on January 24, was a chemist who in twelve years as Managing Director of The Wellcome Foundation, Limited and a further three as a Deputy Chairman was concerned particularly with the profitable translation, through development and production, of the fruits of scientific research into important drugs and vaccines. As President of the Association of the British Pharmaceutical Industry he was a leading figure during an era when regulations were being established in the wake of the thalidomide tragedy. His integrity, relied upon alike by those in industry, medicine and government, was a major factor in the harmonious and successful negotiations. As first-ever Chairman of the Spastics Society he brought together two organisations concerned with the welfare of spastics and presided over a massive expansion on the financial side, making possible the sponsorship of original research on a considerable scale.

Denis Wheeler was born in Bristol on 6 September, 1910, and graduated from Bristol University where he specialised in physical chemistry and carried out research work which led to a Ph.D. in 1934. He was awarded a Salters' Fellowship to study industrial organisation and practice in the United States. Part of the time was spent at Princeton University where he studied advanced physical chemistry. In later years he was to become Master of the Salters' Company and concerned with the continuation of this type of scientific exchange.

On his return to Britain he joined the research department of Imperial Chemical Industries (Explosives) Ltd where he eventually became technically responsible for all acid production throughout the ICI organisation in Great Britain. In 1940 he was appointed research and development director of Hardman and Holden Ltd, chemical manufacturers.

He joined the board of directors of The Wellcome Foundation Limited in January 1946, was appointed assistant managing director later that year, joint managing director in 1948, managing director in 1955 and a deputy chairman in 1967. He retired from the board for health reasons in 1970. He was awarded the CBE in 1965.

In his early years with the Wellcome Foundation, Dr Wheeler was concerned with the organisation of development as a specific function, ensuring that the discoveries of the research laboratories could be produced in the appropriate formulations and on a sufficient scale to be administered to large numbers of humans or animals throughout the world. Whereas the research laboratories of Wellcome had been largely independent of the commercial company, he was able to convince the scientists that their work could still retain its integrity and its impetus whilst being guided towards the discovery of essential medicines, the sale of which would provide the resources for further research.

D. W. Adamson

announcements

Appointment

Brian J. Brinkworth, to the newly created Chair of Energy Studies at University College, Cardiff.

Meetings

4–6 May, **3rd International Congress on Ozone Technology,** Paris (International Ozone Institute, 52 rue d'Anjou, 75384 CEDEX 08, Paris).

8–12 May, **8th International Congress of the International Society of Psychoneuroendocrinology,** Atlanta, Georgia (R. P. Michael, Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322).

9–11 May, **Beltsville Symposium II: Biosystematics in Agriculture,** Beltsville (J. A. Duke, Plant Taxonomy, Room 117, Bldg 001 BARC West, USDA, Beltsville, Maryland 20705).

10 May, **Protein Production: Problems for the Agricultural Engineer,** London (E. Holden, The Institution of Agricultural Engineers, Silsoe, Bedford, UK).

17–21 May, **The Study of Macromolecules by NMR,** Grasmere (The Chemical Society, Burlington House, Piccadilly, London SW1).

18 May, **Opto-Electronics for Measurement and Control,** London (The Institute of Physics, 47 Belgrave Square, London SW1).

18 May, **Animal Health Products—Design and Evaluation,** Groton, Connecticut (D. C. Monkhouse, Pfizer Central Research, Groton, Connecticut 06340).

19–21 May, **The Evolution of Galaxies and Stellar Populations,** Yale (B. M. Tinsley, Yale University Observatory, Box 2023 Yale Station, New Haven, Connecticut 06520).

23–27 May, **2nd European Conference on Marine Technology–Safety at Sea,** London (WEMT 77, 10 Upper Belgrave Street, London SW1).

30 May–3 June, **Ferritic Steels for Fast Reactor Steam Generators,** London (British Nuclear Energy Society, The Institution of Civil Engineers, 1–7 Great George Street, London SW1).

1–3 June, **Environmental Pollutants—Animal Models and Wildlife as Monitors,** Connecticut (G. Migaki, Armed Forces Institute of Pathology, Washington, D.C. 20306).

1–3 June, **31st Annual Frequency Control Symposium,** Atlantic City (J. R. Vig, Fort Monmouth, New Jersey 07703).

7–9 June, **11th Annual Conference on Trace Substances in Environmental Health,** Columbia (D. D. Hemphill, 411 Clark Hall, University of Missouri, Columbia, Columbia Missouri 65201).

Person to Person

A new bivalve mollusc is spreading in the North Adriatic sea; it is similar to *Anadara* or *Scapharca* species except for its greater size (up to 8 cm). Each animal possesses several ml of haemoglobin rich blood. I would be grateful for any information on this topic. Photographs of the mollusc are available on request. Please write to Dr Silvio Buzzi, Felisatti 49, 48100, Ravenna, Italy.

Exchange furnished family house near Littlehampton, West Sussex for similar in Canberra, August 1977–July 1978. Pleasant neighbourhood, near shops, schools, and sea. Brighton 40 min, London 90 min. Exchange of car (Renault 4) also considered. Contact John Thornley, 5 Harsfold Road, Rustington, Littlehampton, West Sussex, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

7–9 June, **Deburring and Surface Finishing,** Chicago (Society of Manufacturing Engineers, 20501 Ford Road, Box 930, Dearborn, Michigan 48128).

18–19 June, **International Symposium on Gut Hormones,** Lausanne (S. R. Bloom, Medicine, Hammersmith Hospital, Du Cane Road, London).

19–25 June, **Inborn Error of Metabolism in Man,** Tel Aviv (O. Sperling, P.O. Box 16271, Tel Aviv, Israel).

20–25 June, **6th International Congress of Lymphology, Prague** (Secretariat: Sokolská 31, 120 26 Praha 2, Czechoslovakia).

20–22 June, **4th International Symposium on Mass Spectrometry in Biochemistry and Medicine,** Lake of Garda, Italy (A. Frigerio, Instituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62-20157 Milan, Italy).

27–30 June, **Annual Meeting of the Genetics Society of Canada,** Manitoba (P. J. McAlpine, Genetics, Health Sciences Childrens' Center, 685 Bannatyne Avenue, Winnipeg, Manitoba, Canada).

27 June–1 July, **7th International Conference on Amorphous and Liquid Semiconductors,** Edinburgh (Secretariat, Centre for Industrial Consultancy and Liaison, 14 George Square, Edinburgh).

3–8 July, **3rd International Congress of Immunology,** Sydney (Secretary, P.O. Box 391, Darlinghurst, NSW 2010, Australia).

4–8 July, **Rhythmic Activity of Fish,** Stirling (J. E. Thorpe, Freshwater Fisheries Laboratory, Pitlochry, Perthshire, Scotland).

6–8 July, **Hot Electrons in Semiconductors,** Denton, Texas (D. G. Seiler, Physics, North State Texas University, Denton, Texas 76203).

11–14 July, **5th International Symposium on Gas Kinetics,** Manchester (UMIST, P.O. Box 88, Manchester, UK).

11–14 July, **International Symposium on Dendrochronology,** London (Organising Secretary, National Maritime Museum, London).

11–15 July, **8th International Conference of the World Association for the Advancement of Veterinary Parasitology,** Sydney (Secretary, CSIRO McMaster Laboratory, P.M.B. No. 1, Post Office, Glebe, NSW 2037, Australia).

11–15 July, **2nd International Conference on The Organometalic and Coordination Chemistry of Germanium, Tin and Lead,** Nottingham (P. G. Harrison, Chemistry, University of Nottingham, Nottingham).

17–22 July, **International Symposium on Macromolecules,** Dublin (Macro Dublin 77, Institute for Industrial Research and Standards, Ballymun Road, Dublin 9, Ireland).

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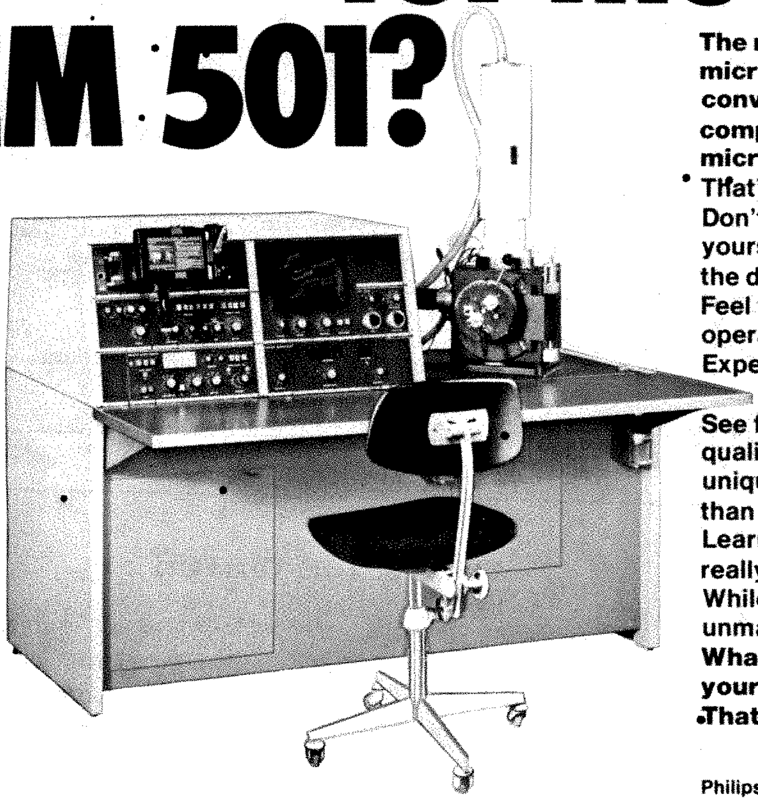

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Scientists think, too

IMAGES of science and the scientist and of engineering and the engineer are much discussed at present. The most recent issue of *Physics Today* contains W. A. Fowler's Presidential Address to the American Physical Society in which some ideas on brushing up the physicist's image are put forward. British engineers, deeply concerned at what they believe is society's lowly rating of them, have been puzzling for some time over how they can better present their profession. And the Spring issue of *Science for People*, the magazine of the British Society for Social Responsibility in Science, is devoted to images of science; we are told that scientific work, being subject to an extreme division of labour, 'tends to be boring, unfulfilling, alienating and unhealthy like most jobs in a capitalist society . . . Being a scientific worker means working in a patriarchal, repressive hierarchy, often in bad working conditions, without control over the fruits of one's labour'.

Images are, of course, needed for a variety of purposes. The student will see the scientific profession in a certain light and will accordingly decide whether or not to contemplate a scientific career. The industrial employer will have a different sort of perception of science related to value to the company. The politician will want to know whether scientists and technologists are basically trustworthy in their advice or whether they are prone to empire building and self-serving pronouncements. The layman, relying more and more on science and technology to deliver the goods, will have his or her own view of whether the scientist and technologist substantiate their claims. Occasionally in all of these directions we can be pleasantly surprised at the reservoir of goodwill, trust and interest which remains.

But there is one image which is in danger of being submerged amidst the recent frenzy to project science as still valuable and scientists as (in Fowler's words) capable, practical, ingenious, innovative and at the same time human beings. That is the image of scientists as thinkers. The public still remembers Einstein and recognises him as a great thinker, but is science in general considered to be a thinking profession? Of course this image is not necessarily one which will be for mass-consumption, but it is an important one nevertheless, because other people in our society most certainly regard themselves as thinkers and it is important that they do not dismiss scientists as doers only, and therefore of not being capable of participating in sensible general discussions on broad matters of policy. This has happened to a large extent in Britain already, with precious little action from bodies which are supposed to have the scientist's interest at heart. There is deplorably little involvement of the scientist in what migh be called 'the life of the nation' and the process is, of course, self-reinforcing: the more the exclusion, the smaller the number of those who can talk sense.

What to do to establish toeholds? Clearly anything involving the expenditure of large sums is not going to be feasible in the near future, so we may have to rule out such desirable ideas as seconding scientists to work with politicians, or the establishment of new interdisciplinary institutions. There still remain, however, opportunities within the media—the arch-purveyors of image. Last year there was great satisfaction that a young biologist should be asked to deliver the BBC's Reith Lectures. But very few scientists are allowed to express their own views in any extended way either on work they are involved in themselves or on more general matters. The formula is generally that scientists, if allowed to speak or write at all for the general public, do so almost invariably through short extracts only, with a journalist or reporter on hand to explain what the scientist means. There is nothing fundamentally wrong with this format (not that many professors in the humanities would stand for it), but in the absence of more extended contributions in the columns of serious newspapers, in magazines and in radio talks, the impression is undoubtedly left that scientists aren't really capable of stringing together more than a few dozen words, are only worth hearing on their very narrow speciality and are best not disturbed in their busy life of doing things. It is time the thinking side of science was given a more public airing. □

Chemical disarmament still elusive

THE Spring session of the United Nations Conference of the Committee on Disarmament (CCD) droned to a close in Geneva this month with little concrete progress made on the thorny issues involved in negotiating a chemical disarmament treaty. Though a proposal put forward last year by the British delegation received many general endorsements—including one from the United States—delegates have been marking time for the past three years awaiting a long-promised but elusive joint proposal from the United States and the Soviet Union.

Part of the problem stems from the fact that there is considerable confusion about the United States position, particularly in regard to the US Army's plans to launch a massive new programme to replace its existing chemical weapons stockpiles with so-called binary chemical weapons. The time has come for President Carter to clarify the matter.

The background is this. In the early 1970s, while US delegates were telling the CCD that the United States supports chemical disarmament, the US Army was requesting funds from Congress to begin building binary weapons—nerve agents made from two non-lethal components which form a deadly compound when mixed together. Not surprisingly, the negotiators were not taken very seriously. In 1974, however, Congress refused to appropriate the funds. Undeterred, the Army came back with another request the following year. This time, the House Armed Services Committee deleted the funds, but it stated that unless progress is made at the CCD talks, future requests for binary weapons would be viewed more favourably.

Consequently, no funds were requested for binaries in the budget proposed by President Ford early last year, but the Army nevertheless argued within the Administration for the programme to be reinstated in President Ford's final budget, presented to Congress last January. Ford decided not to include the funds, however, and passed the matter on to his successor. The binary programme is therefore far fom being killed off, and it still looms large over the CCD talks.

While this was going on, President Nixon and Secretary Brezhnev announced at their summit meeting in July 1974 that the United States and the Soviet Union would offer a joint initiative to break the deadlock at the CCD talks. So far, however, only two rounds of bilateral discussions have been held, the last of which took place early in April, and little progress was made.

Very tricky issues concerned with measures to police a chemical disarmament treaty are involved in the negotiations. But the time has come at least for President Carter to clear the air by announcing that the United States will scrap its plans to produce binary weapons and that the new Administration is serious about chemical disarmament.

The microprocessor phenomenon

Basil Zacharov separates fact and fiction to comment on the microprocessor 'revolution'

SINCE the introduction of the first commercial microprocessor in 1971 by INTEL the growth in number of microprocessors has been little short of phenomenal. It is estimated that the present sales, already £10 million for the European market, will grow at least tenfold by 1980. Microprocessors are used in scientific, domestic, military and commercial applications. Conferences entirely devoted to microprocessors are now commonplace. There are professional journals which are concerned only with microprocessors. And there is hardly any research grant application with a computing or electronic content where the word 'microprocessor' does not appear somewhere or other.

The microprocessor is an electronic device, fabricated on a single small semiconductor chip, enabling certain operations to be performed on input digital data, the transformed data then becoming available either on an output data highway, or in appropriate internal registers of the microprocessor. Depending upon the choice of microprocessor, the width of the data path may be anything from 1 bit upwards, and the operations may include arithmetic instructions, logical instructions and certain others, familiar to every professional computer programmer, such as conditional and interrupt-handling operations. Thus the microprocessor is just one example of a very broad class of monolithic digital elements, using one of several large-scale integration (LSI) technologies to implement as many as 10^5 components on a single semiconductor chip perhaps only 25 mm^2 in area.

Not surprisingly perhaps, the very rapid evolution and exploitation of microprocessors has been accompanied by a corresponding growth in publicity, some of which has been not altogether accurate. There has emerged a mythology about microprocessors or, at the least, a certain amount of confusion. Probably the most widespread myth is that a microprocessor is a microcomputer which, still, it patently is not. Probably one should not be too pedantic about this, and anyway there are many different kinds of stored-programme digital computer, but all of them have to have a memory, a control unit for instruction sequencing and some means of input and output as well as the processing section. So the microprocessor is nothing more than one component of a computer although, admittedly, a very powerful component indeed.

A much more dangerous myth is that it is easy to replace minicomputers by microprocessor systems and presumably, because the cost of microprocessors is so low, that it is possible to build a microprocessor-based minicomputer at a cost much lower than that of commercially-available minicomputers. And so we have seen numerous examples of minicomputers fabricated by the do-it-yourself process. The truth, unfortunately, is that, if all the component costs are really taken into account (including power supplies, circuit cards, casing and so on) and fabrication costs properly included, then it is almost impossible to compete even with the hardware costs of small minicomputers supplied to equipment manufacturers (the so-called 'naked' minicomputers, with no peripherals or other trappings). And this is not the least surprising, for minicomputer manufacturers have been making use of modern LSI components just as much as anyone else. However, if the fact is included that even the most modest minicomputer arrives with some software, then any thought that an individual can compete economically in synthesising a minicomputer just cannot be supported.

A more subtle myth is the claim that, because microprocessors are inexpensive, it is possible to produce at much lower cost a computing system that is comparable in performance to that of large general-purpose computers. Such claims are generally supported by demonstrating that microprocessor-based computing systems can be built with certain instruction execution times comparable to those of some chosen large computer. The more outspoken of those making these claims then go on to conclude that anyone using the large computer on a shared basis would do well to abandon this enslavement and turn instead to a 'personal' computer based on microprocessors. The millennium has, unfortunately, not yet arrived, and the reason is of course clear. It is simply that, although it is indeed possible to achieve comparable execution times for certain operations, as for example adding two integers, this is just hopelessly inadequate to achieve the performance of a large computer for calculations of a general nature.

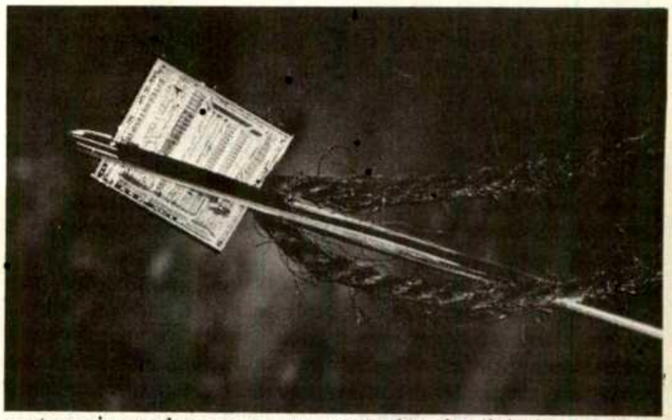
Large computers achieve their performance precisely because of their very extensive repertoire of operations, and also because of their complex internal organisation which allows many different concurrent activities. If this performance is required, then the complexity, and hence cost, of the large system is inescapable. That is not to say that the architecture of modern large computers is the best that can be imagined, nor that a large computer is best for all purposes; just that modern large computers are fairly well matched to the requirements of most large data-processing and scientific calculations, for which a microprocessor-based alternative would be inadequate.

Of course there may be certain special computational tasks that only involve just those operations that can be implemented well with microprocessors; in this case it may well be the best choice to use the microprocessor system. But, even here, the problem has to be one which occurs frequently—otherwise it may still be much more economical to use a conventional computer inefficiently. It must be emphasised here that, just as in the case of minicomputers, large systems use LSI techniques where they can and if they are costly, it is because of all the general facilities that they possess. Microprocessors have no monopoly of LSI technology.

So what is it that microprocessors offer that has led to such an upsurge in their numbers? The most important attributes appear to be small size and low cost, but these relate to the microprocessor as a digital systems component, and not to such things as complete computers. And the advantages in using microprocessors stem from the fact that these attributes enable them to be used either where an application requires large numbers of processors or where processors need to be built into some device or other. Moreover, in applications where the processing task is completely defined and does not need to be changed, there can be a very significant economic advantage in using a microprocessor system tailored to the task rather than use a more versatile minicomputer. The advantages of microprocessors when used in these 'turnkey' systems increase tremendously when the number of similar systems is great. Such problems had been tackled in the past by building special-purpose electronic systems, but these were generally expensive and very difficult to modify. Microprocessors are not only much less costly for these purposes, but also more reliable and, more important, they allow easy evolution from one system to another by changing software.

The real benefit of microprocessors has appeared in computer peripheral devices, especially terminals, and in instrumentation of all kinds. So we have seen a dramatic change already in data presentation from instruments to human beings, and in every application where electronic systems have to respond in an adaptive way to external conditions. This introduction of so-called 'intelligence' into electronic systems has transformed the operation of a wide range of systems, from oscilloscopes to washing machines, from missiles to traffic lights, from computer terminals to automobile electronics. What was costly and complicated to do before using conventional hard-wired electronic components has now become inexpensive and relatively simple to implement.

This trend towards replacement of hard-wired electronic logic by software-programmed microprocessor-based systems is nowhere more apparent than in digital communications, itself one of the most rapidly growing technological fields. Already the use of microprocessors in coupling remote devices to computer networks is commonplace, but we are witnessing only the beginning of this trend. However, all these applications generally involve the use of only a few LSI devices for each unit of equipment, and we need also to remember the other area mentioned, where many microprocessors are used together.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For many years now there has been the idea that what is difficult to do on a conventional sequential-processing computer might be much easier to do on an array of many coupled processors all operating in parallel. Computational problems which either have intrinsic parallelism or can be suitably formulated in terms of some parallel algorithms exist in many different fields, but their solutions have been impeded by the lack of appropriate hardware. Although attempts have been made to implement parallel computer systems using conventional hardware, these attempts have been immensely costly. Now, with the advent of microprocessors, the construction of parallel processing systems has become a real economic prospect and, together with it, the promise of effective solutions to a whole new class of problems.

So, although there is a mythological element in what is being claimed for microprocessors which sometimes distracts certain sectors from the realities of the situation, it is no myth that microprocessors have made a most fundamental impact on many different fields. Microprocessors are already transforming both the construction and the exploitation of an immense range of instruments, appliances and systems, and the signs are that this trend is only a beginning. But perhaps it is as well to end on a note of caution and to mention a final myth—namely that microprocessors are easy to use, and that it is just as easy for hardware designers to employ them as it was for them to design circuits using conventional integrated circuits. What is overlooked here is of course software, which many circuit designers are seeing for the first time.

It is probably the case that, except for a few talented individuals or for very trivial tasks, it was never particularly easy to program a computer effectively in machine code, and not only are microprocessors no exception here but they are also rather primitive. The solution to this problem is well-enough understood, for example by using what are termed high-level techniques to insulate the microprocessor system user or designer from all the details of particular system components. This solution will take a long time to establish since it depends upon agreement about standards and conventions, among other factors, and these do not yet exist. However, even with high-level techniques, it will still be necessary for hardware designers to be familiar with software. Indeed, they will no longer be circuit designers but rather systems engineers. For this to come about, and for the full potential of microprocessors to be realised, we will have to adapt our educational system to meet the new needs of such systems engineers. □

Freedom for whales

The great whales need protection from man. **Ray Gambell** describes the efforts to provide it

THE control of whaling by the International Whaling Commission (IWC), which regulates about 90% of whaling activity throughout the world, has changed very considerably in recent years. There is now a long term management policy aimed at bringing all whale stocks to their most productive levels. Stocks which have been reduced below these levels in the past are given total protection from hunting so that they can rebuild at the fastest possible rate. All management decisions are now based only on the best scientific advice available on the condition and sustainable harvesting capacities of the stocks. Previously the economic arguments of the whaling industry weighed very heavily in decisions taken on the number of whales which were allowed to be caught and the stocks were successively overtaxed to varying degrees.

This major change in management policy started in 1965 but developed more rapidly after the 1972 UN Conference on the Human Environment in Stockholm at which a three-part resolution on whaling was adopted. It called for the strengthening of the IWC, an increase in international research on whales, and a ten-year moratorium on commercial whaling.

Ever since the IWC was set up by the International Convention for the Regulation of Whaling, signed in 1946, the secretariat had been provided on a part time basis by the UK Ministry of Agriculture, Fisheries and Food. In recent years it became clear that the part-time secretariat had difficulty in meeting the increasing demands placed upon it and so in 1976 the Commission established a full-time secretariat headed by an experienced whale biologist. The offices of the Commission have now moved from London to Cambridge, where there are a number of research instituions concerned with whales and other marine mammals. The secretariat is now able to devote itself completely to the development of the Commission's activities, including publicity, publications and the general servicing of the Commission's day-to-day requirements.

International research

A great deal of current knowledge of the biology, life history and status of the stocks of the great whales has been gained with the generous cooperation of the whaling industry itself. Biologists have been able to collect and analyse materials from the whale carcasses during normal commercial operations and facilities for sighting and marking programmes, which would not otherwise have been undertaken have been provided. Inevitably, with the reduction of the whaling industry in recent years, the scope for research based on commercial operations has declined. This is particularly noticeable in terms of the sighting areas covered by the fleets, which now provide the only records of the protected species.

Whale research on an adequate scale for proper global management is very expensive. In 1975 the Scientific Committee of the IWC put forward detailed proposals known as the International Decade of Cetacean Research (IDCR), for research on the distribution and status of the stocks of whales in the southern hemisphere, North Pacific, and North Atlantic Oceans. They have been submitted to the United Nations Environment Programme as one possible funding agency. It is estimated that the first year of this ten-year programme will cost $2.4 million at 1974 prices and, so far there has been no progress in obtaining funding of this magnitude. The delay has been partly brought about by a number of bodies awaiting the research proposals from the Food and Agriculture Organisation (FAO) Mammals in the Sea Scientific Consultation held in Bergen in September 1976.

In the meantime the IWC has set up its own voluntary research fund. Only a few contributions have been received so far, but it is hoped that a number of the IDCR proposals can be funded and implemented through this research fund. In addition, of course, the various national research groups continue their own programmes.

When the IWC considered the call for a total moratorium on commercial whaling it accepted the advice of its Scientific Committee that such action could not be justified scientifically since prudent management required regulation of each stock individually. Nevertheless, the Commission did formulate and adopt precise guidelines for the control of whaling based on the concept of Maximum Sustainable Yield (MSY). All stocks of whales are now classified into one of three categories according to the advice of the Scientific Committee:

- Initial Management Stocks, which may be reduced in a controlled manner to achieve MSY levels or optimum levels as these are determined.
- Sustained Management Stocks which will be maintained at or near MSY levels and then at optimum levels as these are determined.
- Protection Stocks, which are below the level of sustained management stocks and will be fully protected.

All whale stocks have a natural capacity for increase and a natural rate of mortality. At their initial level these two factors balance one another so that the stock remains more or less in equilibrium. As the stock is reduced the pregnancy rate rises, the age at which the whales start to reproduce falls and recruitment increases. At some particular stock level the surplus of recruits over natural deaths reaches a maximum—the MSY—which can be safely harvested without reducing the stock size. At stock sizes below this level the surplus of recruits over natural mortalities declines until another equilibrium position is reached at very small stock levels.

The Scientific Committee has responsibility for identifying stocks of all species of large whales and classifying them according to these categories. The classification is revised annually. The size of catch which may be taken from the stocks is then determined more or less from automatic rules. If a stock is to be judged to be substantially smaller than the MSY level total protection is declared. If it is larger, catching is permitted under quotas which should allow a controlled reduction of the stock towards the MSY level. If the stock is already near the MSY level annual quotas are set with the intention of maintaining the stock at about that size. The rules for determining catch limits allow for possible errors in MSY estimates. For example, no more than 90% of the estimated MSY can be taken whatever the classification of the stocks. This gives a margin of 30% either way in the estimation of the MSY stock level with a symmetrical yield curve. There are also supplementary rules for determining the rates of catch when there is insufficient knowledge to assess the status of the stock, especially when exploitation has only just begun, and also when a stock has apparently been exploited at a sustainable level for many years.

Scientific advice

Despite the advances made by the IWC in bringing its house into order in recent years, there is still considerable public interest and debate over the way in which the whale stocks are harvested. One area of criticism concerns the activities of the Scientific Committee itself. There is much discussion

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Ray Gambell is Secretary to the International Whaling Commission.

over the concept of MSY as a basis for management. The Scientific Committee is very much aware of this problem and is open to any proposals which would help it in its task of assessing, classifying and offering advice on the regulation of the whale stocks. For the moment, however, the MSY concept is one which does give practical guidelines for the management of the whaling industry which can be kept under constant review. It also allows for new factors to be introduced to develop the idea of optimum stock levels.

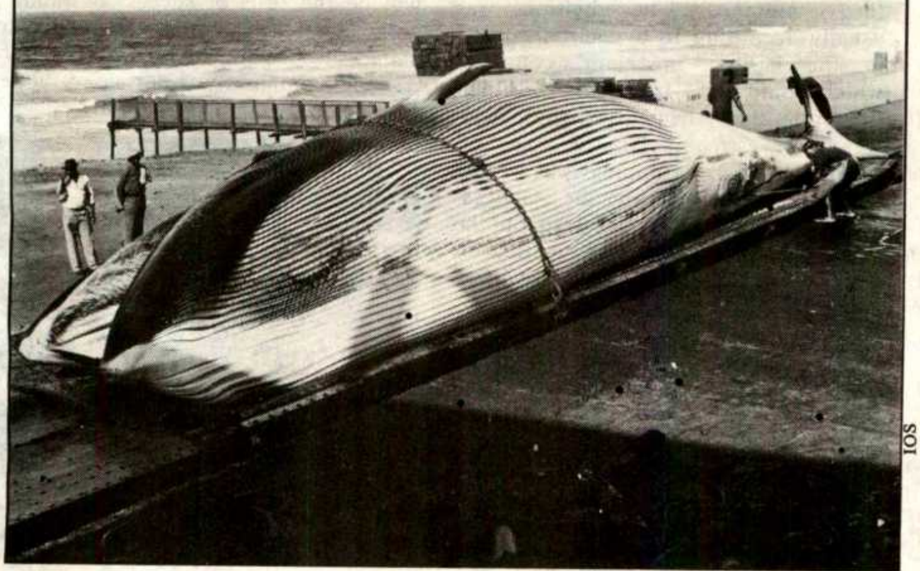
The International Union for the Conservation of Nature and Natural Resources has called for the replacement of the MSY concept by more ecologically based criteria but unfortunately, it is not yet possible to quantify such a basis for management. Discussions are going on between the IWC Scientific Committee and the IUCN with a view to developing these ideas.

A further source of difficulty is the extent to which the Scientific Committee's activities and discussions are available for public scrutiny. Because it meets in the two weeks immediately preceding the Commission's Annual Meeting held in June every year, there is no possibility of publishing the reports of its work before the meeting. This has happened because the Committee must have the latest catch statistics from the Antarctic whaling season which finishes only a few weeks earlier. However, all the scientific papers contributing to management decisions are available immediately after the meeting, and most of them are published subsequently in the Commission's Annual Report.

The Commission has been criticised as a club for exploiters but the majority of the sixteen member nations do not catch whales commercially. Also, any government may adhere to the 1946 Convention and so play a full role in the conservation and management of the whale stocks. When the Commission was first set up it recognised that specialised agencies related to the United Nations would also be concerned with the conservation and development of whale fisheries. It therefore agreed to decide within two years of the Convention's enforcement whether it too should become a UN agency. In 1950 it resolved that it should remain an independent body, but at the same time decided to maintain its close association with the FAO. The question of IWC affiliation to the UN has not since been raised within the Commission.

The 1946 Convention is currently being reviewed and a meeting is being planned of plenipotentiaries from all significant whaling countries or countries with stocks of cetaceans off their coasts to elaborate a new cetacean convention. It is very important that all whaling should be under unified international control, for if unregulated catching goes on there is little prospect for the survival of whales as a valuable resource and component of the marine ecosystem.

Although only some 10% of the world's whaling is carried out by nations which are not members of the IWC, their catch represents all or a major part of the whaling activity in certain parts of the world. For example, off the coasts of Chile and Peru these two nations are the only countries actively whaling, while the Spanish and Portuguese catches in the temperate Atlantic are the only ones in that area. In addition, catches by Korea and a Somali registered vessel overlap with IWC members' operations. The IWC has noted that these activities are seriously weakening its conservation measures and it has repeatedly called on these countries to adhere to the 1946 Convention. So far, diplomatic approaches by individual governments and by the United Nations and various of its specialised agencies have met with a negative response.



Pin whale on the Durban whale track

IOS

International inspection

The Commission's regulations are enforced by whaling inspectors appointed by the flag nation of each catching operation. They report on any infractions and carry out prosecutions through their national jurisdiction. The Commission has also established an International Observer Scheme, whereby observers are exchanged between nations which are in or operate within the same geographical areas. Thus, Japan and the USSR exchange observers on their factory ships in the Antarctic and in the North Pacific. Land station operations in Japan receive observers from the United States, and the Icelandic whaling station has a Canadian or Norwegian observer. A similar exchange is planned between Australia and Brazil, the two Southern Hemisphere land station operations with the IWC.

The observers report directly to the Commission on the manner in which the whaling operations and the inspection schemes are carried out and check that the various regulations governing whaling activities are properly implemented. It has been argued that such an interchange between nations with common interests is not sufficiently independent and that observers from non-whaling nations should be placed at the various whaling factories. This is a matter for international agreement but it must be recognised that the present observer schemes are a significant step forward in the control of the whaling industry on a global scale.

The history of the regulation of whaling this century is generally one of failure. A number of stocks have been seriously depleted in every phase of whaling activity, for whales can be caught more quickly than they are able to reproduce. Nevertheless, the recent advances in the organisation and actions of the IWC make the future look more hopeful. Although there are still many difficulties and many problems of a scientific and administrative nature the present direction of the IWC and its stated intention to manage the stocks in a responsible fashion give cause for future optimism. □

Now, an implementation problem

Robert Stein writes on the United Nations Water Conference which took place last month

The UN Water Conference finished its work on 25 March in Mar del Plata, Argentina, and the ripple was slight. Most of the reports coming out of the conference said nothing much happened, which was taken by reporters on the scene to be either good or bad. That no serious political controversy disrupted this conference in the way that political issues tore apart the end of the UN Human Settlements Conference in Vancouver last June was certainly positive. There was a lot of serious discussion both by those with technical expertise and those concerned with the political implications of the various uses of water. On the negative side, the lack of exciting controversy did not result in very many concrete advances, and this is seen as a failure of the conference since the problems are still waiting to be addressed.

But what was the conference trying to do—and did it come at all close to accomplishing its purposes? The representatives who journeyed many thousands of miles to participate in the conference—over one thousand of them from 116 states—had been given documentation which contained both good and bad news. There was enough water globally but, as Gilbert F. White put it in the basic UN background paper, "Frustratingly, it tends to be available in the wrong place, at the wrong time, or with the wrong quality." Yet for the developed world, the bulk of the problems had until the past few years not been theirs. Pollution, yes, but there was little expectation that a UN conference could help advance their perception of the problems. Then in the summer of 1976, water shortages in northern Europe, and the lack of snow in North America the following winter woke others up to the fact that drought was not merely a problem for the Sahel. Sound water management begins at home.

At the same time this conference, ill prepared as it was (the preparatory committee meeting in January had only one day to review the draft of the plan of action), had a specific charge, provided by the UN Human Settlements: to consider targets for supply of potable water and waste disposal.

Many of the countries of the underdeveloped world addressed water supply as their most pressing problem. Kenya, Tanzania, Nepal, and Pakistan all issued pleas to the conference for help. A number of African countries called for a new special fund to assist countries in water supply and waste disposal—a cry which was not answered. Instead, the conference adopted a resolution calling for a study to figure out how to finance a variety of water development projects, a study which would not exclude the possibility of a fund.

Additionally, and more importantly, the conference approved a resolution put forward by Argentina, the host government, which called upon governments needing improved water supply and waste disposal systems to prepare programmes and plans by 1980 to to reach the Habitat goals of water for all rural and urban areas by 1990. These plans would then be reveiwed to determine how they could best be funded. Interestingly, the problems of maintaining broken-down systems, which are usually ignored, were made an integral part of the recommendations. The resolution was much less specific on which body or organisation would help with the implementation, but that was left for the UN's Economic and Social Council to work out. The conference dealt only generally with problems of water in arid lands and drought, preferring to let the Deserts Conference, this August, tackle that issue.

Management of shared river basins contributed greatly to whatever substantive disagreements took place at the conference. The division was usually according to a country's place in the basin: 'upstream' countries which could hold back water or pollute it to the detriment of those who were 'downstream' banded together, providing such groupings as India against Bangladesh on the Ganges; Turkey against Syria and Iraq on the Euphrates; Brazil against Argentina on the Parańa; and Germany, Switzerland and France against the Netherlands on the Rhine. The resulting recommendation was clearly a political compromise urging cooperation and using the waters of the basin equitably in accordance with principles of international law. Advance consultation was also watered down, with no mandatory requirements. The work of international legal bodies was also encouraged, though given past performances, many disputes—acrimonious as well as peaceable—will arise before these groups conclude their work.

The conference ended positively. An attempt to introduce a statement, which most aptly would have been called the "Declaration of Mar del Platitude", was defeated; instead the recommendations themselves were enshrined. But after the delegates packed their bathing suits and stacks of documents, and left the shining sea and all the plain-clothes police behind, what is next?

UN Conferences can only be a reflection of the willingness of member states to do something. Agreeing to a fund does not necessarily provide any real money to build water supply systems or irrigation works. A conference must—and the UN Water Conference just barely did—supply tools enabling countries and international aid-giving agencies to act in specific directions if they so choose. They are not law making conferences, like the UN Conference on the Law of the Sea is meant to be, nor can they be in two weeks. The proof is in the implementation, a part of the conference process too often ignored both by governments and the UN system.

So the questions remain. How will governments react to the proposals when they are presented to the UN Economic and Social Council in July? How many governments will make efforts—which they otherwise would not have done—over the absence of the water supply programme or the provision of aid and technical assistance to those that need it? How will the conference affect programmes of international agencies such as the World Bank, WHO, FAO, WMO, UNDP, UNICEF and all the others who came to the conference to make sure they weren't cut out of the action? These will be the tests of success or failure, and though it is too early really to gauge success, the record of the international community is not a very hopeful one. □

Robert Stein is Director of the North American Office of the International Institute for Environment and Development

PROFILE

Kenneth Owen on Sir Ieuan Maddock

Bread-and-butter or exotica?

Sir Ieuan Maddock

A CHOICE trio gave details of the Labour Government's new Ministry of Technology at a Millbank Tower press conference in November 1964—Frank Cousins, trade union leader turned minister; C. P. Snow the author, alias Lord Snow, a junior minister; and Professor P. M. S. Blackett, deputy chairman of the minister's Technology Advisory Council. Dr Ieuan Maddock at that time was with the Atomic Energy Authority at Aldermaston. The following September he moved over to Mintech at the invitation of Dr John Adams to help get the fledgling ministry moving.

At the end of last month, six ministers and two ministries later, Sir Ieuan Maddock, FRS retired as Chief Scientist at the Department of Industry. He remains acting director of the National Physical Laboratory.

A tough, ebullient Welshman, he had needed both toughness and ebullience to steer industry and government research establishments towards what he saw as the right kinds of technology. In the process he offended both high-technology industry and the establishments: he was bruised by the expediency of politicians.

The concept of Mintech had been generated primarily by Blackett, who knew at first hand how science had been harnessed to immediate national objectives during the 1939–45 war and who believed that industry could now be regenerated by a similar approach. Maddock joined Mintech as a deputy controller. It was then a very small ministry, responsible for only four industries—electronics, computers, telecommunications and machine tools. Mintech expanded with the transfer of the engineering industry divisions of the Board of Trade in February 1966 and Maddock went on to head five industrial divisions.

Mintech also broadened in scope to deal with basic industrial structures, not just technology. One facet of this approach led to the setting-up of ICL as Britain's national computer company. Various other programmes were mounted to stimulate the application of advanced machines and techniques in industry. "By the summer of 1969", Sir Ieuan recalls, "we were really beginning to get results".

Then came a body-blow—the change of government in 1970 which abolished Mintech and put its initiatives into reverse. "The new government classified everything we were trying to do at Mintech as 'interventionist'. The shutters came down absolutely and completely. You were guilty until you could prove yourself innocent. A lot of schemes were scrapped long before we had a chance of finding out whether they were good or bad".

In his work as Controller of Industrial Technology at the new Department of Trade and Industry (DTI), Maddock was becoming more of an administrator, working on, among other things, the future of the steel industry. "By that time I had moved a fairly long way away from science and technology". This was rectified in February 1971, when he was appointed to the new post of Chief Scientist at the Department. He was to be responsible for the general strategy and coordination of the Department's research activities, while retaining his responsibilities for the DTI sponsorship of the steel, electronics and computer industries.

"At that time", Sir Ieuan says, "I found it personally very difficult. I was dedicated to what we had been doing at Mintech and I was seeing it dismantled. I was having to fight rearguard actions all the way, saying 'Look, you're throwing all kinds of babies away with the bathwater'—and subsequent events proved that to be right.

"I have to confess I didn't enjoy that part of my life at all. In nearly 40 years of government service it is the only period I can recall when I was really pretty unhappy with my environment".

Fresh air blew in with the Rothschild proposals, fully supported by Maddock, which introduced the customer–contractor principle into government R&D spending. "With the dip in my moral factor at that time", Sir Ieuan says, "this gave me something to get my teeth into".

The DTI itself was not a customer department, in the way that Defence was; how should the Rothschild principles be applied? Maddock's solution was to set up a group of industry–government research requirements boards to act as proxy customers, decide priorities and allocate funds. As their programmes came under scrutiny from the boards, the government laboratories hated the whole exercise, Sir Ieuan admits cheerfully. The balance of R&D spending changed, with less being channelled through the establishments and more through the research associations (and through industry).

On public occasions throughout his time at Mintech, DTI and DoI, Sir Ieuan has repeatedly stressed the importance of the bread-and-butter industries and the dangers of pursuing high-technology exotica. The transfer of existing technologies is more important than the creation of new ones; this has become his personal crusade.

"It's not so much the money, it's where you address the best intellects", he says. "The staff list at Harwell in about 1954 contained the cream of the cream; what a hell of a risk that was". The best Japanese brains, meanwhile, were going into the engineering and manufacturing industries. "That's why they're knock-hell out of us now".

Thus Sir Ieuan finds himself "a bit of a renegade" on the Joint European Torus, because "if JET comes to Britain it's going to suck in another new generation of cream talent"—talent that is sorely needed in the engineering industries.

During his time at the three ministries, Sir Ieuan has worked with seven ministers: Cousins, Benn, Rippon, Davies, Walker, Benn again and Varley. His most stimulating time was at Mintech, working with Benn as minister and the formidable Sir Richard (Otto) Clarke as Permanent Secretary.

He quotes two main overall conclusions: First, the tragedy of 1970 when promising Mintech projects were killed off indiscriminately; clearly this still hurts. Secondly, the fact that all the various microeconomic attacks on particular industrial problems will be of little benefit without a stable, healthy macroeconomic background. "Unless you've got that", he says, "technology will not flow".

From 63 to 400?

Colin Norman in Washington examines the implications for nuclear power of last week's energy proposals from President Carter

"THIS has been an exceptional week", President Carter declared last Friday during a packed press conference. Indeed it has. It began with a sombre televised address in which Mr Carter suggested that overcoming the energy "problem" would be a little like waging a war, and ended with a press conference in which he defended his strategy for winning that war. The strategy itself was unveiled on Wednesday, and it has so far achieved the distinction of uniting commentators in their observations that dramatic measures may be needed, but that some of Carter's measures should be resisted.

The strategy consists of a bold set of measures designed to curb Americans' seemingly insatiable appetite for energy by raising energy prices, taxing heavy users, and returning the revenues back into the economy in the form of rewards to people who conserve (see box). The plan is also designed to decouple industry from its reliance on oil and gas by forcing more widespread use of coal, and it also attempts to encourage the use of light water reactors. The emphasis is on the near term.

The proposals for nuclear power are particularly interesting. On April 7, Carter announced that, in the interests of curbing nuclear proliferation, the Administration will defer indefinitely its plans to reprocess commercial nuclear fuel and that it will sharply downgrade the breeder reactor programme. Last week, those decisions were spelled out in more detail, but at the same time, Carter made it clear that the Administration does not intend to turn its back on nuclear power. Far from it. Carter in fact announced that the Administration will seek to remove some of the roadblocks to licencing nuclear plants, step up attempts to find suitable burial sites for nuclear wastes, and completely change direction on uranium enrichment by building a large new enrichment plant using gas centrifuge technology instead of the present gaseous diffusion process.

The intention, in short, is to rely on light water reactors, without plutonium recycle, to meet a growing share of energy needs for at least the next 25 years, but to keep the breeder reactor effort ticking over as insurance against the possibility that the United States will need to move into the plutonium economy early in the next century. According to officials of the Energy Research and Development Administration (ERDA), the number of light water reactors in the United States is expected to grow steadily from the present figure of 63 to between 300 and 400 by the turn of the century.

The nuclear strategy rests on some critical assumptions, however, perhaps the most important of which involves the size of the United States' uranium reserves. Proven reserves now amount to about 1.8 million tons, which would be sufficient to fuel about 300 reactors throughout their lifetimes—barely enough to meet ERDA's projections for domestic nuclear expansion this century, with none left over to meet contracts from abroad. ERDA is, however, launching an intensive programme to assess uranium reserves more accurately, and acting ERDA Administrator Robert Fri said last week that he is "guardedly optimistic" that proven reserves will swell to about 3.7 million tons. There is some question, however, whether the electricity industry, faced with uncertainties in long-term uranium supply and with no guarantee that plutonium recycle will be available to help stretch nuclear fuel supplies, will be enthusiastic about purchasing nuclear plants.

As for the breeder reactor programme, there is still some confusion about the Administration's intentions. One thing is certain, however: the effort is being sharply cut back and it no longer occupies pride of place as the top priority programme in the United States' energy research and development effort. Last week, Carter proposed that the programme's budget be reduced by $173 million next year, in addition to a cut of nearly $200 million which he proposed in February. The plan now is to complete the fast flux test facility, a test reactor now under construction in Washington, but to defer indefinitely plans to build a large demonstration reactor on the Clinch River in Tennessee. That project, which was to have cost more than $2,000 million, will be redirected to focus on alternative breeder reactor designs which present less of a proliferation hazard. The exact nature of the programme has not yet been

The central feature of President Carter's energy strategy is a carrot-and-stick arrangement of rewards and penalties designed to break the country's addiction to vast quantities of energy. The carrot comes in the form of an assortment of tax breaks and other financial incentives for people who insulate their homes, buy small cars or install solar heaters. The stick is a complex set of proposals which will lead to higher energy prices, and the threat of stiff taxes on gasoline and on gas-guzzling cars. The goal is to reduce the growth in energy demand from its present level of about 3.5% a year to about 2%.

Coupled with those efforts to stimulate conservation is a series of proposals designed to increase the use of coal and light water reactors. Longer-term energy supply options will also receive modest increases in research support, but the emphasis is mostly on efforts likely to produce relatively quick results.

The major elements of the plan:

Oil and gas prices: A complex set of taxes coupled with price increases for newly-discovered domestic crude oil would cause oil and gas prices to increase until they "generally reflect the true replacement cost of energy". At present, prices are kept artificially low by a variety of controls.

Gasoline taxes: Unless gasoline consumption is reduced according to specific targets, tax increases of 5 cents per gallon will be imposed each year, beginning in 1979, until the total tax reaches a maximum of 50 cents.

Gas guzzler taxes: New cars which fail to meet gasoline consumption standards would be heavily taxed, and the revenues would be returned in the form of rebates to people who buy more efficient cars. For example, 1978 model cars which get less than 12.5 miles per gallon would carry a tax of $449, while those which get more than 39 miles per gallon would be given a rebate of $473. By 1985 cars with those consumption levels would carry taxes and rebates of $2,488 and $493 respectively.

Insulation and Solar heating: All new buildings will have to meet insulation standards. Homeowners who insulate their houses would be entitled to income tax credits of 25% of the first $800 and 14% of the next $1,400 spent on conservation measures. Similarly, people who install solar equipment before 1985 would be able to claim 40% of the first $1,000 and 25% of the next $6,400 as a tax credit.

Nuclear power: Light water reactors without plutonium recycle will have an increasingly large role. New gas centrifuge plants will be built to enrich uranium for reactor fuels, more funds will be pumped into efforts to find suitable waste disposal sites, and an attempt will be made to reduce the present 10-year licencing process for nuclear plants to 3 years.

Coal: Many industrial facilities would be forced to switch from oil and gas to coal, and they would also be required to use the best available technology to control pollutants. Research and development on processes designed to produce synthetic oil and gas from coal would be stepped up. In addition, Carter will establish a commission to report by October this year on the health effects associated with mining and burning large quantities of coal. And a new research programme, costing $3 million, will be launched to look into the so-called greenhouse effect.

worked out, however, and Administration officials suggest that it will be weeks, if not months, before it is.

As for the plan to build a large centrifuge enrichmenmt plant, this again is coupled with Carter's moves to curb nuclear proliferation. In 1974, the United States closed its order books for long-term supplies of enriched uranium to other countries because of projections that US enrichment capacity would be used up. The move is said to have prompted many countries to seek alternative sources of supply, and it may have been a key factor in persuading Brazil to negotiate the purchase of an enrichment plant from West Germany. Carter has therefore announced that the United States will re-open its order books, and that a supply of enriched uranium will be guaranteed to those countries which renounce the development of nuclear weapons.

To meet the projected demand, a large centrifuge enrichment plant will be constructed at a cost of some $4,400 million. So far, all US enrichment has used the gaseous diffusion process, and previous plans called for expansion of an existing diffusion plant at Portsmouth, Ohio. Administration officials say, however, that the gas centrifuge process, which has been developed in secrecy at Oak Ridge, Tennessee, is now ready to be commercialised. So far, however, no site for the new plant has been chosen, though hints that it may go to Oak Ridge, to compensate for cancellation of the breeder reactor project there, have sparked loud protests in Portsmouth.

In addition to relying on a modest expansion of nuclear generating capacity, Carter's plans for medium-term energy supply also envisage considerable expansion in the use of coal. He has proposed measures to force industry to convert from oil and gas to coal, announced large increases in research and development efforts aimed at producing synthetic oil and gas from coal, and recommended a number of regulations to reduce the environmental consequences of such an expansion.

There is, however, one potential environmental problem which could prove intractable: the so-called greenhouse effect which may lead to changes in the global climate from large increases in atmospheric carbon dioxide. Carter announced last week that a major study of the greenhouse effect, costing $3 million, will be launched this year.

Carter's energy plan will now be picked over on Capital Hill, and already special interest groups are clawing at it. The debate is likely to rage for the rest of this year. ☐

GEOS

Hope from despair?

Stuart Sharrock in Washington and Chris Sherwell in London report on the ill-starred launch of Geos, the European Space Agency's scientific satellite

On Tuesday of this week a group of space scientists and another of space engineers met separately on opposite sides of the Earth to discuss an event that occurred above the globe halfway between them. Last Wednesday, twenty minutes after a Thor-Delta 2914 rocket had taken off from Cape Canaveral only 0.2 seconds later than its scheduled lift-off of 05.15, the hopes of 11 research institutes from eight member countries of the European Space Agency turned to despair as a malfunction during the separation of the second and third stages of the rocket caused the third stage to separate early. It meant that the payload, the world's first geostationary scientific satellite known as Geos, could not achieve its planned orbit and that seven experiments which took years to prepare might no longer be done.

In fact the despair looks as though it may have been premature. For although the engineers from the rocket manufacturers McDonnell Douglas gathered in California to discuss the Delta's acknowledged failure, the scientists who met at the ESA's Space Operations Centre at Darmstadt in West Germany did so a little more optimistically. On the previous day Geos had been placed in an elliptical orbit whose apogee, at 38,498 km, was easily as high as the originally intended circular orbit.

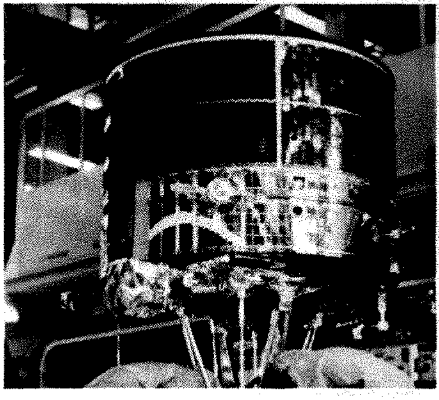
The difference, of course, was that the new orbit, not being geostationary, would not allow the satellite to be permanently within sight of the Darmstadt ground station. It is understood, however, that not all the data obtained over each 24-hour period would have been used anyway. The new elliptical orbit, selected to maximise the return of scientific data, has a perigee of 2,131 km and an inclination of 26.85°. It is traversed every 12.06 hours by the satellite, which could now be within sight for some 10 hours a day.

As the scientists gathered to decide which experiments to pursue and data to collect, ESA officials were pleased to have retrieved, if only partially, a mission which at first looked on the very edge of disaster. The spacecraft and third stage assembly should have spun up to 97 revolutions per minute before separating from the second stage. This spin stabilisation would have ensured that the third stage had an accurate trajectory during its burn; without it, the spacecraft tumbled around into the wrong orbit, though it was stabilised soon thereafter.

The spacecraft could not have been allowed to stay indefinitely in this so-called transfer orbit because it went through zones of high radiation which might endanger the experiments and decrease the total active lifetime of the spacecraft. To stay in that orbit also served no useful purpose: it was not on the magnetic field lines connected to the auroral zones of the Arctic and Antarctic, and the experiments would probably not work with the field and particle intensities so high.

The original intention was that the spacecraft should be injected into an elliptical transfer orbit (apogee 35,500 km, perigee 230 km) and then be boosted on the fourth apogee to a circular orbit synchronised with the Earth below. That the European-built solid-propellant apogee booster motor responsible for this task placed Geos in the elliptical orbit it now occupies is a cause for comfort, even though the motor could not be used twice to achieve a circular orbit. But then so much could so easily have gone wrong.

The effect, ironically, is that Geos is now placed in a fashion that had been expected of the ESA's spare 'qualification' geosynchronous satellite which was due to be sent up in 1979 atop a European Ariane launcher now being developed. It remains possible that this back-up satellite will be upgraded to become the 'true Geos', but it will cost the ESA another 13 million units of account to purchase the launch from NASA—a cost which is not recoverable for last week's launch under NASA's no-loss-no-profit contracts. Although the ESA Council is due to meet next week, it is unlikely to discuss this idea of inverting the roles of the two satellites. ☐



Quarks out of the bag?

In a paper given at the American Physical Society Spring Meeting in Washington DC, Drs W. M. Fairbank, A. F. Hebard and G. S. LaRue of Stanford have presented evidence for the existence of fractional electric charges. The experimenters emphasise that they are claiming the existence of fractional charge; they do not mention quarks.

Their experiment is a sophisticated version of the classic Millikan oil-drop apparatus. A superconducting niobium sphere 0.25 mm in diameter is floated in a magnetic field and its net residual charge deduced by analysing its behaviour in an oscillating electric field, the net charge can be altered by integer values of the electron charge e, by using a radioactive source. Fractional charges of $\frac{1}{3}$ e or $\frac{2}{3}$ e carried by a quark would be detected as the sensitivity claimed is 1% of the electron charge.

The most severe background is electric and magnetic dipole moments on the spheres which mimic the effect of a fractional charge. These dipole backgrounds are now understood and have been eliminated, according to the experimenters.

Of eight spheres measured, two show evidence of fractional charge: the first was seen last September and the second in mid-February. One had a measured residual charge of minus 0.331 ± 0.070 e which went to zero after handling at room temperature. A second sphere has a charge of plus 0.337 ± 0.009 e. It has been measured on three occasions and the charge remained after the sphere was taken to room temperature.

Preparation of the spheres includes heat treatment during which five spheres were supported by niobium and three by a tungsten substrate. The two spheres showing fractional charges came from the tungsten substrate set.

Why have all other quark searches been fruitless? In an interview with *Nature*, Dr Fairbank argued that searches have only been carried out on a limited variety of materials. If quarks were released in extremely energetic events during the early stages of the universe one can speculate that they go through some unknown evolutionary process to end only on heavy elements.

Initial reaction to the announcement is firstly surprise that quarks could be so abundant as to show up on two out of only eight spheres, and secondly amazement that an antiquark is seen. The experiment cannot distinguish a residual charge of $+\frac{1}{3}$ e from $-\frac{2}{3}$ e.

If these quarks are genuine they will challenge much recent theoretical work. Present understanding of quarks inside protons is that they are light and bound within the proton—to remove one would require an infinite amount of work. Heavy free quarks could be accommodated within the theory but they would be very different objects from those inside the proton and much of the aesthetic beauty of the theoretical framework would be lost.

Future plans of the Stanford team include measuring more spheres, investigating the significance of their mode of production and evaporating the material to separate and collect the charges.

Stuart Sharrock

IN BRIEF

Brenner's appointment

The UK Medical Research Council (MRC) has announced that Dr Sidney Brenner will become leader of the MRC Laboratory of Molecular Biology (Hills Road) in Cambridge on the retirement of Dr Max Perutz in 1979.

The news is little surprise for those who have heeded the rumours that followed an internal MRC report produced last year on the future of Hills Road, suggesting a radical change in organisation. The MRC has followed this, for whereas Dr Perutz was chairman of the Governing Board of the laboratory, Dr Brenner is to be its Director.

The Governing Board at present consists of Dr Perutz, Dr Hugh Huxley (whose appointment as Deputy Chairman has also been announced), Dr Brenner, Dr John Gurdon, Dr Cesar Milstein, Dr Fred Sanger and Dr John Smith. It has been collectively responsible for the running of Hills Road, with no particular power being vested in the chairman.

In 1979, however, the Governing Board will probably be disbanded. Its replacement, if any, is currently under discussion, but whatever the outcome Dr Brenner is certain to end up with a good deal of power. What is more, several of the senior staff at Hills Road will have left by the time Dr Brenner becomes Director. Dr Francis Crick has recently announced that he is to remain at the Salk Institute where he is currently on sabbatical, and David Blow has just taken up a chair at Imperial College, London. It is also rumoured that that two other senior members, both on the Governing Board, have their eyes on other posts.

Another novelty for Hills Road is the fact that it is a shrinking laboratory. The staff is to be gradually pruned by 25%, back to its 1972 level.

All told Sidney Brenner will have a considerable challenge on his hands if he is to maintain the world-wide reputation of Hills Road during the course of his Directorship, which automatically lasts (as does the laboratory) until 1987, when he is 60 years old. Dr Brenner was recently heard to say "I am going downhill and I am going with a lot of noise". Only the second part of that statement is true.

Atkinson for NSF

Richard Atkinson, a former professor of psychology at Stanford University, has, as expected, been named by President Carter to be Director of the US National Science Foundation (NSF). Generally regarded as an effective administrator well versed in dealing with Congress, Atkinson has been Acting Director of NSF since August 1976, when the previous incumbent, H. Guyford Stever, departed for a brief stint as science adviser to President Ford. Atkinson was deputy NSF director for 17 months before that. A top-flight researcher in fields ranging from the development of memory to the application of computers to instruction, Atkinson is the first social scientist to be appointed Director of NSF.

Whose power to choose?

There is now "a unique and possibly unrepeatable opportunity" to bring international action to redirect the worldwide use of nuclear power, according to Brian Johnson, author of a paper published by the International Institute for Environment and Development, *Whose Power to Choose?* At a time when President Carter is committed to controlling nuclear proliferation and the US nuclear industry gets 70% of all nuclear power station orders, there should be the opportunity, he argues, to bypass the plutonium economy by assuring countries of fuel for the thermal reactors they need, safeguarding the international community from proliferation and creating programmes to develop alternative energy sources.

To implement such proposals he suggests a reformed IAEA: its Department of Safeguards and Inspection should be strengthened, all nuclear facilities should be licenced with the IAEA to ensure security and environmental safety, and it should enforce internationally agreed measures for waste disposal. A new energy agency could be developed, independent of the United Nations but with support from OPEC and the US, to look into ways of helping poor countries shed their dependence on oil and nuclear power by developing alternative energy sources.

Uranium for Europe

President Carter's statement in last week's energy message to the US Congress that it was time "to resume uranium enrichment services" to Europe and elsewhere is likely to be well received in the European Community. The Nine's dependence on imported energy and on nuclear power was stressed heavily by the President of the European Commission, Mr Roy Jenkins, when he met Mr Carter earlier in Washington. Mr Jenkins told Mr Carter that without adequate and assured supplies of enriched uranium, European countries would be more tempted to invest in reprocessing and fast breeders.

Supplies from the United States were held up pending a review of US policy on nuclear exports: a total of almost 2,900 pounds of uranium, for various destinations around the world, was involved. Canada, Europe's other main supplier, has held up supplies too, pending full agreement on safeguards and nuclear exports.

Preparing for 1979

The ten members of the Committee on Science and Technology in Developing Countries (COSTED)—a grouping established within ICSU in 1966—gathered in London for a couple of days last week for their annual meeting. Among other things, they are understood to have discussed their position in relation to the forthcoming United Nations Conference on Science, Technology and Development, taking the view that a non-governmental group of scientists ought to participate in their individual capacities. COSTED also hopes to help in the preparation of papers by developing countries.

UK vacancies

During 1976 the British Civil Service had vacancies for about 340 scientific officers at grades up to Senior Principal Scientific Officer. About 11,000 applications were received, but even so only 250 appointments were made, the Civil Service Commission says in its annual report. It seems that recruitment of scientists is a particularly tricky business; as many as 50% of those made offers decline to take them up.

Public expenditure cuts obliged a number of major departments to withdraw entirely from recruitment in the second half of the year.

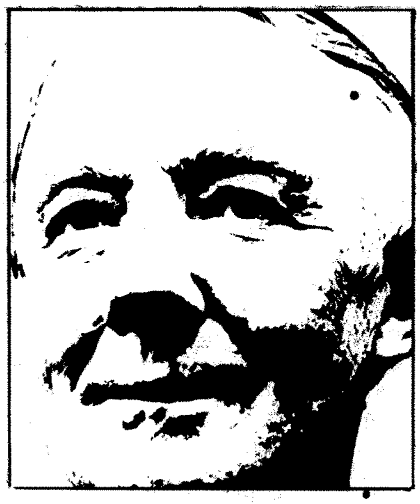
Concorde's noise

A gloomy report on Concorde noise levels has emerged from a working group of the Noise Advisory Council (HMSO, 50p). So gloomy in fact that the Secretary of State for the Environment, who is chairman of the council, specifically states that the views expressed are those of members of the council only and not of the Secretary of State.

The first operational figures for Concorde noise at Heathrow were obtained in the summer of 1975. They showed take-off noise to be on average several dB above Heathrow's statutory day-time limit of 110 PNdB at certain monitoring prints. The working group were told that an improvement of a few dB could be expected in cooler weather and when pilots became more experienced.

Operational figures for eight months of commercial operations are now available. The working group consider there has been little or no improvement on the 1975 figures, and suggest that the only way to improve Heathrow's noise climate would be to ban Concorde at night and in the evening and early morning, to restrict other jet flights at night still further, and to require Concorde operation to balance its increased noise with reductions in flights of other noisy aircraft.

Pollution economics



KENNETH MELLANBY

DURING the past ten years many writers have suggested that global pollution is the most serious hazard facing mankind. They say that if we insist on economic growth and further industrialisation and on using more energy, catastrophic environmental degradation is inevitable. This was the message of *The Limits to Growth* and *A Blueprint for Survival.* It would be foolish to deny that pollution is a problem, or that new industries may give rise to serious damage. We should welcome attempts to regulate developments such as those set out in "Suggestions for the development of a hazard evaluation procedure for potentially toxic chemicals", a memorandum from the Monitoring and Assessment Research Centre (MARC) at Chelsea College, London. But we should also recognise that many new techniques are much less polluting than those which they replace, and that any alleged correlation between the use of energy, or a rise in GNP, with pollution, is spurious.

In fact, changes are often in the other direction. London's air is cleaner in 1977 than it was in 1877, though the population has increased and far more energy is consumed. In their recent book, *The Next 200 Years,* Herman Kahn and his colleagues of the Hudson Institute suggest that vastly increased economic growth and energy consumption will be needed to ensure global cleanliness. The same view is, in effect, held by the British government. Thus drastic measures are set out in the Control of Pollution Act passed by Parliament in 1974 which was intended to activate the law to reduce all types of environmental pollution. However, many of the most important sections of the Act have not yet been brought into operation.

It is generally agreed that, in Britain as in most industrialised countries, river pollution is the most serious problem. We know how our fresh waters could be improved, and we have the methods to do so. Yet it is just those provisions which would most affect our rivers which are in abeyance, and this is because our economic record is so dismal and our growth so slow. Mr Denis Howell, on behalf of the Secretary of State for the Environment, confirmed this in *Hansard* on 17 February, when he informed Parliament that "Implementation of the remaining provisions [of the 1974 Act] has been delayed because of present constraints on public expenditure but will take place as soon as possible".

This lack of legislative progress is disappointing, but is it important? All the evidence suggests that, during recent years, our dirtiest rivers have continued to improve even without the force of the new law. Every year more species of fish return to the Thames, and we now have a report that there is a healthy population of three-spined sticklebacks in the River Clyde off Glasgow Green—the first instance of fish breeding in this area for over a hundred years. Even with their limited resources authorities with the will to succeed have found a way to improve the situation. To restore salmon and trout to the Thames and the Clyde will take time, and the expenditure of a great deal of money for more and better sewage treatment. Only industrial growth or a drastic reorganisation of our economic system can supply the funds. To try to enforce regulations for which money is not available is a recipe for chaos, not for a clean environment.

correspondence

Scottish science

SIR,—Martin Goldman's distaste for the idea of devolution in Scotland (17 March, page 208) should not let him mislead readers by saying that Scotland does well out of grant-giving bodies such as the Medical Research Council (MRC).

The MRC spends 8.6% of its budget in Scotland which has 9.7% of the UK population (*Hansard* 18 December 1975) and if the figure were based on the number of medical students or teaching staff it would be 17%.

I am told by those who advise the MRC that the reason the Scottish figure is low is that Scottish applications are rare, and that the reason for this is that the Scots believe that the clever people in London are more deserving of the money. The only cure for this affliction of the spirit is devolution or federalism.

Finally, Dr Goldman's view that devolution or federalism cannot work will be read with amusement in North America.

Yours faithfully,
D. N. M. HAMILTON

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Nuclear irrationality

SIR,—My brief essay "Nuclear Power, Public Interest and the Profesisonal"[1] was essentially a plea for critical rationality (and the consequent anti-authoritarian approach) in the way society evaluates nuclear power. Two letters[2,3], present critical comments which I think are partly irrational.

Dr Grantham makes several misleading statements. One example is the comment on small reactors and "bomb-capacity commercial reactors" in relation to "a few microgrammes (of plutonium) can kill you". Another example is "the (nuclear) battle is one-sided. Pronuclear propagandists are paid: the opposition is not". Apart from pointing to the falsehood in the latter part of this statement, I emphasise that I do not look at the nuclear debate as a battle. My essay was an attempt to get at the facts and to emphasise the need for a rational debate on all "sides".

Dr Grantham has also chosen to disregard completely almost all my basic points. He states that "nuclear industry representatives seem to want to exclude biologists, environmentalists and the general public from the controversy", and "instead of trying to restrict the discussion to experts we should indeed extend it to everyone". In this connection I refer to two of my statements: "Probably no technological decision in the history of mankind has been the subject of so many detailed studies, so much open discussion and such broad participation", and "The scale and timing of the introduction of nuclear power is a major and complex decision. In a democratic society such decisions are not left to technological experts but to elected political officials".

Dr Grantham objects to what he calls my "suggested dichotomy: reasonable, rational and valid" versus "irrational, emotional and ethical". Again a quote from my essay is relevant: "Clearly, a number of irrational, emotional and ethical factors may be and should be of importance in a choice of power plants for the production of electricity".

On the role of scientists in public debate, Dr Anfinsen states that "anyone but the most naive petition-signer must understand that sponsors of socio-scientific news releases are, and always will be, selected on the basis of the fact that the general public tend to be influenced by lists of the names of 'dignitaries' ". This is precisely the basis for my criticism of petition signers using their titles when these are irrelevant to the matter. For example, a responsible *nuclear* physics professor will know if his personal background justifies him speaking on the *nuclear* energy question as a *nuclear* physicist (with a consequent intended and justified substantial impact on politicians and the public), or if he ought to speak as Mr J. Q. Public. Hence my plea that specialists should not mislead the public by carrying their specialist background as a flag in unrelated issues.

Incidentally, in referring to E. Rabinowitch (for whom I have great respect) as "a person concerning whose judgement and expertise in the area of science and public policy no one would quibble", Dr Anfinsen appears to embrace precisely the authoritarian approach which parts of my essay were directed against.

For Dr Anfinsen, as for myself, the question of a connection between nuclear power and potential nuclear wars is a key issue. It deserves not only the religious and emotional considerations put forward by Dr Anfinsen and the National Council of Churches of Christ in the USA, but also our utmost rational efforts to reduce the risk of such wars, with or without nuclear power. I fail to understand the rationality of distracting public attention from the all important issue of nuclear disarmament by expending substantial efforts on stopping the peaceful uses of nuclear power, which probably decrease rather than increase the risk of war.

Finally, I offer, upon Dr Grantham's request, my definition of rationality: to be rational and reasonable is to apply flexibly the control of our knowledge and our theories to our actions, so that we can perform the continuous trial and error process required for improving our adaptation to the environment by way of critical argumentation, thus reducing the need to subject our physical beings to those vagaries of nature and emotions which are harmful.

The place of facts in the world of human values should be set by the values, but facts cannot be changed by values.

Yours faithfully,
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[1] Doderlein, J. M., *Nature* **264**, 202 (1976)
[2] Orantham, R., *Nature* **265**, 100 (1977)
[3] Anfisen, C.B., *Nature* **265**, 492 (1977)

Requests for reprints

SIR,—Would you publish an appeal to scientists throughout the world on a matter which is on the one hand extremely trivial but on the other could eliminate a nuisance. I refer to the request for reprints by fellow scientists. Besides the cost of mailing these reprints, it is time consuming to cut out addresses from post cards, glue them onto envelopes, decipher poorly written names and very often have to write the whole address by hand or typewriter. It would make life so much easier if requests in future carried a stick-on label as a few from considerate colleagues already do.

Yours faithfully,
F. ECKSTEIN

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news and views

Formation of planetary atmospheres

from Robert Hutchinson

THE inner planets are usually considered as having formed by the accretion of solids at least part of which could release a volatile fraction when heated. Outgassing from hot interiors would then have led to the formation of atmospheres. To understand the development and evolution of a planet's atmosphere therefore, the bulk chemical composition of the primitive material from which the body was built must be established. This is not an easy task; the bulk composition of the best known planet, Earth, is still disputed today.

During the past 10 years, cosmochemists, largely led by Anders' group at Chicago, have found that many meteorites are composed of fractions each representing stability at a particular temperature/pressure condition. Heterogeneity in meteorites led to the idea of heterogeneous accretion of planets (Harris & Tozer *Nature* **215**, 1449; 1967; Turekian & Clark *Earth planet. Sci. Lett.* **6**, 346; 1969).

Essentially, the Earth is thought to have formed by growth of a small body composed of refractory Fe–Ni metal and/or Ca–Al–Ti–rich oxides and silicates. As it grew, lower temperature Mg–Fe silicates were added until, lastly, accretion ceased with the addition of a hydrated, low temperature veneer. Heating of the veneer released water for our oceans and gases for our primitive atmosphere. Like many theories in planetary science, heterogeneous accretion is not without its critics. And the largely qualitative treatments of the 1960s are now being quantified.

It is against this background that the paper by Rasool and Le Sergeant in this issue of *Nature* should be viewed (page 822). The Viking landers obtained an estimate of the chemical composition of the martian atmosphere, so there is now reliable information on the atmospheres of two inner planets which must be accommodated in any model of atmospheric development. Using the simplest treatment, Rasool and Le Sergeant assume the terrestrial and martian atmospheres to be essentially closed systems. They begin by looking at the better known Earth and attempting to find a suitable source material for the volatiles. Carbon- and water-rich carbonaceous chondrite meteorite is rejected because the Earth's atmosphere has too much ^{20}Ne and other heavier inert gases relative to C (and H). Instead, the volatile-poor LL group of ordinary chondrites is chosen as it gives a better match, especially for the inert gases. It is argued that a 116-km thick veneer of LL group chondrite could have been outgassed to produce oceans and atmosphere.

When a similar model is applied to Mars it is found that the quantity of LL group chondrite required to produce the atmospheric ^{36}Ar content would produce more N, C and H than are observed in the atmosphere. The amounts of ^{84}Kr and ^{132}Xe agree with the ^{36}Ar abundance. Rasool and Le Sergeant go on to argue that if the N, C and H are not in the atmosphere, they may be in the regolith or on the martian surface; for example, H may be present as polar water ice up to 40-m thick. Near surface volatiles could be temporarily released to form an atmosphere of CO_2 and H_2O with surface pressure of 400 mbar.

Finally, the high ^{40}Ar content of the martian atmosphere is considered in the light of the low upper limit (0.25%) for K in the martian surface (^{40}K is the radioactive parent of ^{40}Ar). Because the martian surface is apparently not as enriched in K as is terrestrial continental crust (but compare oceanic tholeitic basalt, with less than 0.1%K) Rasool and Le Sergeant argue that ^{40}Ar on Mars has come from a greater thickness of material than the other volatiles. It is also implied that K has not been concentrated upwards on Mars due to lack of complete melting in this planet.

It is likely that Rasool and Le Sergeant's paper will prove to be controversial. A probable line of attack is that terrestrial O isotopic ratios define a chemical fractionation line enriched in ^{16}O relative to LL group (and other) ordinary chondrites (Clayton *et al. Earth planet. Sci. Lett.* **30**, 10; 1976). This argues against an LL group origin for terrestrial volatiles, but for the foreseeable future, martian O isotopic ratios will remain unknown. ☐

Redox potentials of iron-sulphur clusters

from A. J. Thomson

THE non-haem iron proteins consisting of clusters of iron and sulphur are known to be involved in a wide range of biological processes from steroid hydroxylation and CO_2 and N_2 fixation to oxidative phosphorylation and photosynthesis. They have been isolated from obligate anaerobic bacteria, aerobic bacteria, algae, plants and all animals so far investigated. Their single most important function is the catalysis of oxidation/reduction reactions and the transport of electrons to reaction centres.

For this reason the range of redox potentials exhibited by the Fe-S clusters is of great interest. It is now emerging that proteins containing a common structural Fe-S centre can have widely differing redox potentials. For example the high potential iron protein (HIPIP) from *Chromatium vinosum* and the bacterial ferredoxin from *Peptococcus aerogenes* are close structural relatives. The former contains a single cubane-type four-iron sulphur cluster $[FeS_4\text{-(S–Cys)}_4]$ whereas the latter possesses a pair of these units. However, functionally they are very different. HIPIP in the oxidised form gives an EPR signal which is abolished on reduction, and the mid-point potential of this process is +340 mV. By comparison the bacterial ferredoxin exhibits a one-electron reduction also but to give an EPR signal in the reduced state only and with a mid-point potential of about −400 mV. These puzzling observations were neatly explained by a hypothesis due to Carter *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **69**, 3526; 1972) which suggested that the clusters in the two proteins were undergoing reversible redox changes between two different oxidation states, in the following manner

$$\underset{\text{HIPIP oxidised}}{\overset{\overset{C^{-}}{\text{(paramagnetic)}}}{[Fe_4S_4(S\text{-}Cys)_4]^{-}}} \rightleftharpoons \underset{\substack{\text{HIPIP reduced}\\ \text{Ferredoxin oxidised}}}{\overset{\overset{C^{2-}}{\text{(diamagnetic)}}}{[Fe_4S_4(S\text{-}Cys)_4]^{2-}}}$$

$$\rightleftharpoons \underset{\text{Ferredoxin reduced}}{\overset{\overset{C^{3-}}{\text{(paramagnetic)}}}{[Fe_4S_4(S\text{-}Cys)_4]^{3-}}}$$

It is not therefore to be expected that the two proteins will show similar mid-point potentials or EPR characteristics. The three-state hypothesis was quickly given confirmation by Cammack (*Biochem. biophys. Res. Commun.* **54**, 548; 1973) who showed that HIPIP could be further reduced by dithionite to the C^{3-} state termed the super-reduced form of HIPIP in the presence of more than 70% by volume of dimethylsulphoxide which serves to unfold the tertiary structure of the protein. This demonstrated that the protein environment prevents further reduction or oxidation of the cluster, ensuring that only single electron transfers are carried out by the protein. Subsequently it was shown that in the presence of ferricyanide the bacterial ferredoxins can be super-oxidised to produce an EPR signal similar to that of oxidised HIPIP (Sweeney *et al. Biochem. biophys. Res. Commun.* **59**, 188; 1974).

Although crystal structures are available for both HIPIP and the bacterial ferredoxin from *Peptococcus aerogenes* it is not clear what features of the protein environment control the redox potential of the cluster. In the past eighteen months two new examples of iron–sulphur proteins have been investigated which show that the control by the protein environment is remarkable. The first example is a protein from *Azotobacter vinelandii*, FdI, which has been obtained in crystalline form and shown to contain two four-iron clusters. (Sweeney *et al. J. biol. Chem.* **250**, 7842; 1975). As isolated the protein shows an EPR signal very similar to that from super-oxidised bacterial ferredoxins. The signal disappears on photoreduction with spinach chloroplast fragments with a mid-point potential of −420 mV. Apparently the cluster is undergoing the C^{-} to C^{2-} reduction with a mid-point potential ~ 760 mV below that of the HIPIP cluster. Furthermore, oxidation of FdI with ferricyanide produces an EPR signal on uptake of one electron but with a mid-point potential of +350 mV. It is concluded that *A. vinelandii* FdI is the first example of an iron–sulphur protein which contains two clusters functioning between the same pair of oxidation states as HIPIP but with mid-point potentials 760 mV apart.

The second, and more recent, example is seen in three ferredoxins (FdI, FdI′ and FdII) extracted from the sulphate reducing bacterium, *Desulphovibrio gigas* (Cammack *et al. Biochim. biophys. Acta* **490**, 311; 1977). With molecular weights of 18,000, 18,000 and 24,000, respectively, the three proteins appear to be oligomers of a basic unit of molecular weight 6,000 containing one four-iron cluster. By monitoring the appearance and disappearance of the C^{-} and C^{3-} states with low temperature EPR measurements the mid-point potentials of clusters within the three proteins have been determined. Their values are summarised below.

	FdI	FdI′	FdII
$C^{-} \updownarrow C^{2-}$	$\updownarrow$ −50 mV	$\updownarrow$ −30 mV	$\updownarrow$ −130 mV
$C^{2-} \updownarrow C^{3-}$	$\updownarrow$ −455 mV	$\updownarrow$ −130 mV	$\updownarrow$ −437 mV

The three ferredoxins differ markedly in the intensities of the various EPR signals leading to the conclusion that FdI undergoes reduction between C^{2-} and C^{3-} states, FdII between C^{-} and C^{2-} states and FdI′ can be produced in both C^{-} and C^{3-} states. There is no evidence that a single cluster can reversibly change between all three oxidation states. A more plausible interpretation is thought to be that in some subunits of the protein the four-iron centre undergoes the C^{-}/C^{2-} transition whereas in other subunits of the same protein the C^{2-}/C^{3-} transition can take place.

Although the three-state hypothesis undoubtedly correctly assigns the oxidation states to the clusters, the very property it sought to explain—the difference between the redox potentials of HIPIP and bacterial ferredoxins—requires more subtle interpretations in the light of these new results. Several mechanisms for the control of the cluster redox state by the polypeptide have been discussed. It has been shown that the cluster geometries in oxidised and reduced *Chromatium* HIPIP are significantly different. This led to the suggestion that the size and shape of the cluster-binding cavity might be designed to favour one oxidation state. (Carter *et al. J. biol. Chem.* **249**, 6339; 1974). Alternatively, a given oxidation state could be stabilised appreciably by the formation of hydrogen bands between the cluster and the protein. Carter and coworkers (*op. cit.*) have identified NH+ . . . S hydrogen bonds in their crystal structure determination of HIPIP. Finally, it may be significant that the newly discovered examples are all of proteins containing more than one cluster. It could be, as Sweeney, Rabinowitz and Yoch suggest, that inter-cluster interaction is strong enough to modify mid-point potentials. The study of water-soluble model compounds could provide some interesting comparative data.

New light on old mesons

from Peter J. Litchfield

The excitement generated in the high energy physics community by the discovery of the ψ/J mesons and the emergence of the 'new physics' of charm has tended to overshadow developments in the field of conventional meson spectroscopy. However, new results from a partial wave analysis of the reaction $\bar{p}p \rightarrow \pi^{+}\pi^{-}$, just published by a Queen Mary College, Daresbury Laboratory and Rutherford collaboration (*Phys. Lett.* **67B**, 117; 1977), have shown that the old-fashioned charmless physics still has some surprises in store. The groups have determined the spins and parities of three resonances with masses approaching those of the ψ mesons; by some way the heaviest conventional mesons with well-established quantum numbers. The states seem to lie on a daughter Regge trajectory with unexpected properties which, if confirmed, could cause a major upset in our theoretical ideas. The minimum disruption would require a modification of the predictions of Regge models at high energies and momentum transfers. It could, on the other hand, be possible that these resonances are manifestations of four quark 'exotic' states and signal the opening of a whole new branch of particle physics.

The search for high mass resonances is complicated by their small branching fractions into any given final state and their large widths which result in strong overlapping of adjacent resonances. The only satisfactory experimental access to them is by way of formation experiments in which measurements of the differential cross sections and polarisation parameters are made as a function of centre-of-mass energy. This enables the reaction to be decomposed into its constituent partial waves and thus enables resonances in a given spin parity (J^{P}) state to be observed. The new discoveries have been made possible by high statistics measurements of the differential cross sections and polarisations for the reaction $\bar{p}p \rightarrow \pi^{+}\pi^{-}$ in the centre-of-mass energy range 2,020 to 2,580 MeV made at CERN by the QMC, Daresbury and Rutherford groups (together with physicists from Liverpool for the differential cross section data). They are the first measurements which are sufficiently precise to enable a definitive partial wave analysis to be performed in $\bar{p}p$ annihilations. The analysis has revealed the presence of resonances at masses of 2,150, 2,310 and 2,480 MeV/c^2 with spin parities 3^-, 4^+ and 5^- respectively. The two lower mass states have been observed before as bumps in the total and elastic cross sections but this is the first determination of their quantum numbers.

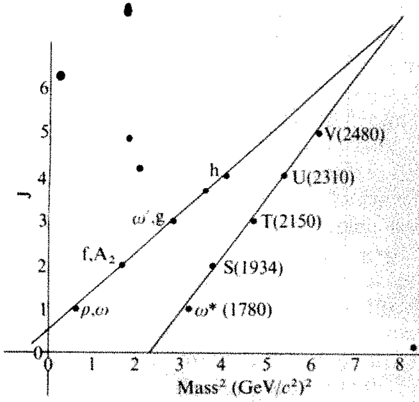
One method of classifying resonance states is to plot them on a graph of spin *versus* mass squared where states are found to lie on straight line trajectories known as Regge trajectories. Figure 1 shows this plot for the dominant mesons found in production experiments, the ρ, ω, f, A_2 . . ., together with the three new states, another pp resonance found at a lower mass (the S(1934)) and a 1^- state recently reported from an e^+e^- annihilation experiment at Orsay. There are clearly two separate trajectories, one for the dominant mesons in production and one for the dominant mesons formed in pp annihilation experiments.

The plot yields two surprises. First, the lower trajectory (called a daughter trajectory) is not parallel to the leading trajectory. Regge phenomenology has always assumed that trajectories are linear and parallel. Although very closely linear, the slope of the daughter trajectory is considerably greater than that of the leading trajectory. This could have important consequences if the trend continues since at higher energies and momentum transfers the new trajectory would dominate the ρ trajectory which is the only one usually retained in theoretical calculations.

Second, it is puzzling that the dominant resonances in $\bar{p}p \rightarrow \pi^+\pi^-$ are not on the leading trajectory as is the case in all other known reactions. An observation by this same group from their data on $\bar{p}p$ elastic scattering taken as a by-product of this experiment (*Nucl. Phys.* **B113**, 1; 1976), indicates a possible explanation. In that reaction they find that there is a characteristic maximum radius of interaction of approximately 1.5 fm. If this is also true in the $\pi^+\pi^-$ reaction and we assume a simple impact parameter model where the angular momentum is given by the radius of interaction times the centre-of-mass momentum, then the maximum angular momenta accessible at the resonance masses are those on the daughter trajectory. Resonances on the leading trajectory could not be formed.

An alternative but much more speculative explanation is that the new states are qualitatively different from those on the ρ trajectory, that is, they are made up of two quarks and two antiquarks rather than only one of each. Speculation on the existence of such exotic states has been rife in the theoretical world for several years and there has recently been rather weak evidence that such states may be formed in e^+e^- annihilations around 4.03 GeV (see *Nature* **266**, 301; 1977) and in $\bar{p}p$ annihilations at 12 GeV/c (*Phys. Lett.* **66B**, 185; 1976). Theoretically pp formation experiments would be the ideal place to observe such states since an interaction in which a quark–antiquark pair was annihilated from the three quarks in the proton and three antiquarks in the antiproton would leave a state consisting of just two quarks and two antiquarks.

$\bar{p}p$ annihilations have long been advocated as the place to search for high mass mesons. It is only now after many years of effort that data of sufficient quality to realise these expectations have been obtained. Many questions remain. Does the new trajectory continue linearly and overtake the leading trajectory, or does it eventually flatten out? Data at higher masses could give the answer. Better data on the K^+K^- channel (also measured in this experiment but with much lower statistics) or on the 3π channel would give access to states not available in the $\pi\pi$ channel. Do they have similar properties to those described here? Does the $\bar{p}$ just have a propensity to interact at a range of about 1.5 fm or are these states truly exotic? The interest of high energy physicists in conventional meson spectroscopy has been reawakened and already new experiments to investigate the $\bar{p}p$ system have been proposed. ☐



Picornavirus RNAs are interesting . . . and unique

from T. J. R. Harris

Apart from satellite tobacco necrosis virus RNA, picornavirus RNA molecules provide the only well established exception to the 'capping' rule. These messenger RNA molecules lack the $m^7G(5')ppp(5')X$. . . that is characteristic of so many virus specific and cellular mRNAs from eukaryotic cells (see *Nature*, **263**, 188; 1976).

Experiments with polynucleotide kinase, an enzyme from T4-infected *E. coli* which adds ^{32}P from $\gamma^{32}P$ ATP to 5′ OH groups, initially suggested that the 5′ end of poliovirus RNA was pAp. The possible endonuclease contamination of the kinase preparation used made this experiment rather unsatisfactory and the discovery of a 5′ cap on various virus mRNA molecules (particularly those of other animal viruses which have a genome that is a messenger RNA, Sindbis virus for example) led to a re-examination of the 5′ terminus of poliovirus RNA, as well as that of other picornavirus RNAs.

Several papers have been published in the past year reporting the outcome of these new investigations. It is clear that the 5′ terminus of 35S poliovirus-specific mRNA isolated from the polyribosomes of infected cells is pUp. On the other hand neither a nucleoside diphosphate nor a capping group was found on the RNA extracted from virus particles. As the genome RNA presumably acts as a messenger RNA to initiate infection, this was a distinctly puzzling result. It is not surprising that an answer to the apparent paradox was soon forthcoming. Wimmer's group (Lee *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 59; 1977) and Baltimore and his co-workers (*Proc. natn. Acad. Sci. U.S.A.* **74**, 961; 1977) now have evidence that there is a small protein (molecular weight around 7,000) covalently linked to the 5′-nucleoside diphosphate. As well as explaining the difficulty of finding a 5′ end, the presence of the protein on nascent RNA chains in the replicative intermediate has led both groups to speculate on a role for the protein in RNA synthesis, although some function in virus particle morphogenesis is not discounted.

Another interesting structural feature of picornavirus RNAs, unique among animal virus RNAs, is provided by the RNA of viruses belonging to the cardiovirus sub-group. Like some plant virus RNAs such as tobacco mosaic virus (TMV) and turnip yellow mosaic virus (TYMV), cardiovirus RNAs can be aminoacylated *in vitro*; mengovirus RNA with histidine and encephalomyocarditis virus (EMCV) with serine. The part of the RNA molecule which is aminoacylated has not yet been determined. In contrast the RNA of poliovirus and foot and mouth disease virus (FMDV), both members of different picornavirus sub-groups, cannot be aminoacylated.

Although not containing a capped 5′-end, RNA molecules from representatives of all the picornavirus sub-groups possess the 3′-terminal poly(A) characteristic of a number of other virus mRNAs. In addition to poly(A) the RNA of the cardioviruses contains a poly(rC) tract. Until recently the location of this homopolymeric region in the RNA was not known. Two papers published at the end of last year now indicate that the poly(C) is very near the 5′-end of both EMCV RNA and FMDV RNA and there is the possibility that the sequence is 5′-terminal.

It will be interesting to determine the structural basis of these differences in the RNAs of the various picornaviruses and to discover their functional significance in the replication of the viruses. ☐

Magnetostratigraphic pitfalls

from George Kukla and J. D. A. Zijderveld

CONTROVERSY is growing over the existence of young excursions of the Earth's magnetic field such as the Gothenburg event (Morner & Lanser *Nature* **251**, 408; 1974). Are these features real? Are they true records of magnetic anomalies? Do they influence climate (Fairbridge *Nature* **265**, 431; 1977)? Or are they merely a product of overlooked natural or artificial strata deformations (Thompson & Berglund *Nature* **263**, 490; 1976; Opdyke *Earth planet. Sci. Lett.* **29**, 238; 1976)? The recently reported detailed study of Koobi Fora formation in Kenya (Hillhouse *et al. Nature* **265**, 411; 1976) shows that the magnetic reversal stratigraphy of Pre-Brunhes formation is not without difficulties either. With all these problems, can palaeomagnetic 'magic' still be taken seriously?

The participants* of the steering committee session of Project 128 of the International Geological Correlation Program concluded at a meeting in Paris, last September, that it can. The project, a joint venture of UNESCO and of the International Union of Geological Sciences was organised to foster intercorrelation of magnetostratigraphic zones with regional stratigraphic systems and radiometrically dated levels. It is a continuation of former IGCP Project PA.70.17 (see Kukla & Nakagawa *Geology* **3**, 704; 1975).

But there are complicating features to which increased attention should be paid. The most important of these are the presence of post-depositional magnetostratigraphic overprints, natural deformation of the strata under investigation, deformation caused by the coring and sampling devices and the continuity and sedimentation rates of the deposits.

Finding the remanent magnetisation of a sample is only one of three equally important procedures in magnetostratigraphy. The other two are—establishing the original position of the sample at the time of deposition, and the correlation of recognised magnetozones with the standard palaeomagnetic time scale.

Unchanged position of studied layers

The remanent vector of samples can only be related to the direction of the Earth's magnetic field if the original orientation of the layer is preserved or the amount of later deformation known. Most researchers assume that their oriented samples precisely represent the layer at the time of deposition and dedicate lamentably little effort to testing whether this is really the case. In studies of secular magnetic variation and of magnetic anomalies, this is a critical limiting factor. Unfortunately, the occurrence of local deformations due to slumping, crust loading, mudflows, and so on, even in generally well stratified horizontal beds is common. Such features can be detected in exposures (Verosub *Science* **190**, 48; 1975) but easily overlooked in cores. The coring operation in unconsolidated sediments further increases the incidence of unrecognised deformations. Arching, twisting, and 'sucking in' of sediment frequently affects the cores as can be observed on samples from laminated deposits. Also non-vertical penetration of the corer and systematic inclination errors affect the reliability of data. It is difficult to detect a deformation of homogeneous unstratified layers, although X-ray screening may prove helpful.

Plastic deformations may frequently occur at lithologic boundaries such as sand–clay interfaces. The core may also be deformed in handling, cutting in segments, and in storage. Freezing of cores as practiced by some palynological laboratories can produce large ice crystals which can damage the core structure. In this context, it looks suspicious when all the magnetic excursions reported in the upper 30 m of Biwa Lake core (Yaskawa *et al. J. Geomag. Geoelectr.* **25**, 447; 1973) come from the top or bottom parts of defrozen core segments.

We would not like to imply of course that all local excursions of the Earth's magnetic field reported from cores are misinterpreted records of deformed layers. However, it is only fair to state that at present, no firm evidence exists to the contrary.

Only results reproduced in parallel sections should be taken as indicative of the behaviour of the Earth's magnetic field (Creer *et al. Earth planet. Sci. Lett.* **31**, 37; 1976). A layer with anomalous, but internally inconsistent magnetic record should be first checked in detail for anomalous sediment structure, sliced and X-rayed for example, before any attempt is made to interpret it as a record of past magnetic field. Duplicate or triplicate sampling of a core should be attempted whenever the diameter is sufficiently large to allow such a procedure. Performance of coring devices should be tested on recent sediments of similar consistency and size distribution as the studied layers.

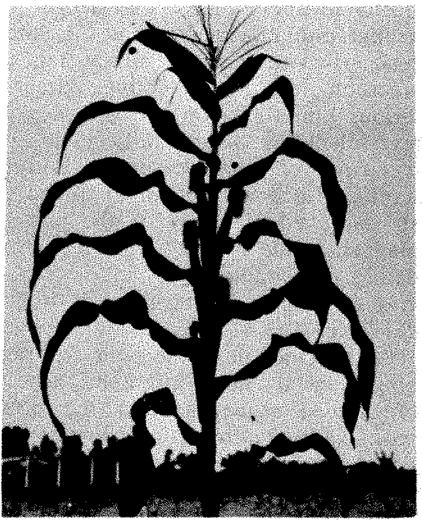
Recovery of original magnetic record

The Koobi Fora study (Hillhouse *et al. op. cit.*) is an excellent example of problems surrounding the recovery of original remanent magnetisation. No universal palaeomagnetic technique is available at present that can guarantee a complete removal of post-depositional magnetic overprint and unambiguous identification of depositional polarity. This could become a serious problem in land-based deposits exposed to prolonged weathering and to the action of groundwater. Sediments deposited during intervals of reversed polarity may acquire fully normal remanent magnetisation during subsequent weathering episodes. Normal remanence of land-based weathered strata does not exclude their formation at the time of reversed polarity. Similar problems caused by remagnetisation were also indicated at the seafloor (Watkins *et al. Earth planet. Sci. Lett.* **24**, 113; 1974). Thus, the polarity corresponding to the time of

*F. P. Bonadonna, N. Bonhommet, E. von Braun, K. Creer, K. Kitazawa, G. Kukla, N. Opdyke, N. D. Watkins, I. Wendt, J. D. A. Zijderveld.

Maize in Yugoslavia

from D. O. Hall



A QUARTER of Yugoslavia's arable land is planted to maize, which vies with wheat as the single most important crop in Yugoslavia. A visit to the Maize Research Institute outside Belgrade proved an eye opener as to how a State Research Institute can be entirely self-supporting from the commercial sale of its products and expertise and at the same time support an active and directed basic research programme which complements its commercial and plant breeding sections. As a novice in this area I may have been over-impressed by the single-mindedness and enthusiasm of the workers at this Research Institute, applied to all stages of the production of a single crop—from research on seed production and gene banks to the extension services on the farm and the sale of seed and expertise (at home and abroad). The increasing interest in growing maize in the UK and Europe both for forage and grain has highlighted the versatility of this plant. It can be grown in a wide range of climates—all that is needed is to find a variety which suits the particular area and the purpose for which it is to be grown.

The Maize Research Institute at Zemun-Polje, about 15 km outside Belgrade, is situated on about 300 hectares of productive land, which is unfortunately now being encroached on by urban and industrial development. Modern well equipped laboratories have a pleasant working atmosphere in extensive fields around a central complex. The Institute employs about 250 people, a quarter of whom have university degrees, and is organised into three different divisions, a science division, an extension service, and an administrative and commercial affairs section. The extension service, which is involved in the controlled production of seed on large cooperative farms and which advises experimental farms, is the crux of the Institute's success. Nearly all the Institute's finance comes from royalties on the sale of its hybrid seeds, both in Yugoslavia and abroad. Nearly a half of its income is from abroad and it has close commercial and research links with more than 20 different countries. Seeds are produced on about 20,000 hectares of good seed-producing land with a suitable climate.

The variability of climate in Yugoslavia from Mediterranean to Continental, its varied geography, and centuries of maize growing, have resulted in a unique maize gene bank. It is claimed that the Maize Institute has more than 100,000 different genetic combinations available for selection of new maize types. The storage of this gene bank is a major part of the Institute's service, since it provides a source of material not only for its own breeding programme but also for research work and hybrid seed production abroad. Yugoslavia seems to have been fortuitously placed in a favourable position for restocking the world's depleted gene banks, especially in North America where the widescale use of hybrid species has resulted in neglect of other varieties selected over the centuries. The Maize Research Institute is making the most of this advantage. Its sales of hybrid seed abroad and patented rights of seed production extend to 18 countries and these hybrids are now grown on over 1.5 million hectares abroad—not bad going for an Institute that only started to specialise in 1959.

Maize in Yugoslavia is mainly fed to cattle. More than 87% of the corn is converted into meat and Yugoslavia is a meat exporter. However fluctuations in the price of meat are resulting in a reassessment of the use of maize and new developments from the United States such as the production of glucose/fructose syrup and the utilisation of the whole maize plant for other types of product are being actively investigated. The potential for greater use of maize seems to be there. Over the past seven years the average yield of maize in Yugoslavia has doubled as a result of the use of the Maize Institute's hybrids which are now grown on two thirds of the total land area cropped to maize. They claim now that they have more than 75 new hybrids which will yield about 10 tonnes of grain per hectare as opposed to the average in Yugoslavia of 4 tonnes per hectare.

Research at the Institute is divided between four sections. Breeding and Genetics naturally employs the largest number of scientists, many of whom have been trained in North America. Research in the Physiology, Biochemistry and Technology Section takes several basic approaches which may have long term application to genetics and maize production. Low temperature growth in maize is being studied in the hope of extending the growing season by earlier planting and of understanding the physiology of low temperature adaptation. Developmental physiology and adaptation to the various climates found in Yugoslavia are also under investigation. Protein biosynthesis is being studied with the aim of increasing the lysine and tryptophan content of the plant. Biophysical assays which can be used for selection during breeding programmes are also being developed. The third section, Maize Protection, deals with fungal and bacterial diseases, insect attack and weed problems. The fourth, Agronomy, is involved in the techniques of maize cultivation. The integration of basic research and seed production and commercial exploitation seems in the case of this Institute to have been particularly successful.

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deposition is to be considered an interpreted property of unequal accuracy, dependent on lithology, intensity of remanence, post-depositional history of the layer, the demagnetisation programme and the instruments used.

In these circumstances, the validity of magnetostratigraphic zonation of sedimentary rocks is only proved by reproduction of the results in sequences involving different lithologies, depositional environments and sedimentation rates. Results of highest confidence are those obtained on stratigraphic successions of effusive volcanic rocks where the danger of remagnetisation is minimal. Magnetostratigraphers are urged to duplicate sampling in parallel sections and in parallel cores wherever possible. Further effort is also needed in studies of rock magnetism dedicated to recognition of original remanence from overprint and to improvement of our understanding of processes through which the remanence of sediments is acquired and destroyed.

Correlation of magnetozones with palaeomagnetic chronology

Magnetostratigraphy is not a dating technique. It only provides globally synchronous correlation planes, whose identification is possible by comparisons with other stratigraphic or radiometric data.

Local polarity zones represent valuable stratigraphic units independent of palaeomagnetic chronology which should be locally labelled and their boundaries clearly identified with respect to lithologic or biostratigraphic zonation. They could, but need not be correlated with the standard palaeomagnetic time scale. The time represented by the deposit is critical in the correlation. Is the deposit continuous? If so, what is the sedimentation rate? Does the thickness of the magnetostratigraphic unit fit the length of palaeomagnetic interval with which it is correlated? Does the reversal occur in a continuously deposited sequence? Or is it only an unrecognised hiatus? Many otherwise excellent magnetostratigraphic studies paid only marginal attention to the continuity and homogeneity of the sedimentary record. This resulted in serious miscorrelations, such as mistaking events for epochs (see Kukla & Nakagawa *op. cit.*).

The palaeomagnetic chronology scale is not a stratigraphic system since it was constructed from radiometrically dated samples of known polarity mostly without any knowledge of stratigraphic relations among the dated horizons. Under these circumstances, precision of K-Ar dating imposes serious limitations on the accuracy of reversal dating. Uncertainty about the true age of most reversals is substantial. Concentrated effort aimed at an improved accuracy of the palaeomagnetic scale by multiple radiometric dating of suitable layers in the stratigraphic vicinity of reversals is highly desirable.

As it stands now, the poor coverage of geological and stratigraphic aspects of studied sequences and the often insufficient removal of post-depositional overprint seem to be the principal weaknesses of magnetostratigraphy correlation. Most of the existing problems could be avoided or at least substantially reduced if repetitive sampling of parallel sections becomes a regular practice. ☐

Plants and animals interact

by Mary Lindley

An international symposium on Biochemical Aspects of Plant and Animal Coevolution was held by the Phytochemical Society of Europe in Reading on 4–6 April, 1977. The proceedings will be published by Academic Press.

CATALOGUERS of the chemical compounds found in plants are being stimulated to new thoughts by ecologists. They are looking for an explanation of how the compounds serve the plants and affect their interactions with animals. The members of the Phytochemical Society of Europe are closely involved and if evolutionary trends are slow to emerge, there seems to be no shortage of enthusiasm and ideas.

Volatile compounds are proving to be important in the pollination of orchids of the genus *Ophrys*, which resemble female insects. Male bees are induced to effect pollination while trying to copulate with the flowers, and some of the stimuli are odoriferous, as G. Bergström (University of Gothenborg) explained. He does not discount visual and tactile stimuli, but it seems that the morphology of the flower plays a small part in the process of attraction. It is not yet known whether the various volatile compounds identified in the flowers enable them to mimic the sexual attractions of the bees—more structures have to be determined, particularly of cyclic terpenes.

The bees gain no nutritional benefit from *Ophrys*, but most of the interactions discussed involved the consumption of plants by animals, chiefly insects. Discussing the role of nitrogen in such interactions, S. McNeill (Imperial College Field Station, Silwood Park) reviewed evidence of a correlation between the abundance of insects on plants and the amount of nitrogen available as protein and amino acids. For example, the most numerous populations of insects on the grass *Holcus mollis* coincide with the peaks of nitrogen during spring and autumn. At the best of times the amount of nitrogen in plants is low compared with that of animals, and insects use various strategies to minimise the disadvantage. When it is lowest they may simply consume more material or move to a more nutritious plant or part of a plant. They might be able to modify the local physiology of the plant, for example by causing proteolysis, or they might turn to occasional predation on other animals as a source of nutrition.

The migratory and desert locusts (*Locusta migratoria* and *Schistocerca gregaria*) select the plants to give them the necessary nutrients through the effects of phagostimulants and deterrents. R. F. Chapman (Centre for Overseas Pest Research, London) explained that sugars, for example, stimulate locusts to feed, while such compounds as alkaloids deter them. It seems that for a hungry locust the choice between different plants is determined by the presence of deterrents, while the size of the meal is more likely to be determined by the phagostimulants in the chosen host. Chapman suggested that two events in the evolution of plants particularly influenced the evolution of the locusts and their grasshopper relatives, the Acrididae. The first flourishing of the Acrididae seems to have coincided with the development of the flowering plants and the decline in the occurrence of condensed tannins, which are powerful deterrents. Later, with the coming of the grasses, the Acrididae could diversify again. Such examples, however, are unlikely to have been common in the evolution of insects, which Chapman stressed, has not been due on the whole to the influence of plant chemistry.

E. A. Bell (King's College, London) said that it has been a tenet of faith for him that plants make so many secondary compounds because they cannot run away from their enemies. The toxins in seeds include lectins, cyanogens, alkaloids and uncommon amino acids such as canavanine, but they are not all equally toxic to all animals. D. H. Janzen (University of Pennsylvania, Philadelphia) pointed out that the protection of seeds often needs to be more effective than that of leaves. Seeds contain a store of nutrients that makes them very attractive to predators unless unpleasant toxins are also present. To study the response of insects to various seed components, Janzen has been using synthetic seeds made from crushed seeds of the animal's host plant and incorporating into them toxins from other plant species.

On the basis of field tests, T. J. Mabry (University of Texas, Austin) believes that sesquiterpene lactones serve primarily as a bitter principle, discouraging animals from feeding. Deer and rabbits, for example, will not feed on plants containing such toxins, which occur most abundantly in the Compositae.

Participants did not all accept the view that secondary plant compounds always have a defensive role. D. A. Jones (University of Hull) feels that there is no absolute advantage in cyanogenesis, by which a cyanogen and the appropriate enzyme release hydrogen cyanide when the plant is crushed. His evidence was obtained with the polymorphic species *Lotus corniculatus* (bird's foot trefoil), which contains both cyanogenic and acyanogenic plants. Jones has found that on a cliff the greater tolerance of salt spray by acyanogenic plants is balanced by the higher potential rate of reproduction of cyanogenic plants. In close proximity, in a hollow and on a mound, selective grazing by snails gives an advantage to cyanogenic plants. Clearly the situation is not as simple as it once seemed.

The traditional idea of a battle between insects and plants is also too simple according to H. F. van Emden (University of Reading). Drawing on his experience with aphids, he presented an alternative viewpoint. Insects such as aphids, which are restricted in their selection of host plants, might gain an advantage if the secondary compounds in the plants are primarily a defence against vertebrate predators. One benefit would be that both the insects and their food would be protected. □

Materials science and engineering

from Robert W. Cahn

"A GENERAL survey or reconsideration" is one of the meanings attached by the Shorter Oxford English Dictionary to the word *Review*. That usage dates from 1604, when modern science was in its birth throes. The natural philosophers of that time knew and corresponded with each other, and that curse and blessing of our day, the scientific journal, was as yet unknown and unnecessary. Today, the general survey or reconsideration is as essential to the working scientist as the Good Food Guide to the serious gourmet.

A review, of course, tells a scientist in a nutshell what the current state of knowledge is in a field, with extra emphasis on recent facts and insights. Who ever heard of a survey of ignorance? Yet that is what the editor-in-chief of the journal *Materials Science and Engineering*, together with his Advisory Board, set out to provide to mark the tenth anniversary of the journal's publication. The 264 pages of volume 25, dated September 1976, are made up of 35 short essays devoted to "what we do not know about" a range of subjects which fall within the scope of the journal. The tacit objective seems to be to guide the research worker who is contemplating a change of field, or of emphasis within a field, into fruitful problems. The editor invited a number of specialists all over the world to map the gaps and chasms in their knowledge. The result is unconventional, variable and useful.

One contributor complains: "Throughout my professional life I tried to teach my students, when they were writing their first paper, not to elaborate too much what they didn't know. Of that, I told them, the future readers know enough themselves." However, the wise choice of expert contributors renders this review of ignorance acceptable and indeed useful. It is necessary to know a great deal before the gaps in that knowledge can be a source of wisdom. Nobody attempted to restrict himself to a survey of gaps: all, with greater or lesser terseness, first surveyed what is known. As another contributor expressed it—"I take it that we are asking: What principles governing . . . are generally agreed by the scientific community? What principles are controversial? What areas are unknown to the extent that no principles have been formulated?" This contributor achieved something very rare and indeed paradoxical in a scientific review: he included not a single reference to published work, but filled his own prescription in a discursive way, emphasising the important points without documentation. The essay — L. M. Brown's on the Deformation of 2-phase Alloys—is a thorough going success. This approach can be followed successfully only with a widely familiar topic; one which is less familiar may need plenty of documentation, and yet effectively pinpoint the crucial areas of ignorance; D. A. Vermilyea's essay on Corrosion is a case in point.

Some reviews are highly theoretical and can only be followed by a reader who is already highly expert (for instance, H. E. Cook on Continuous Transformations; M. Wuttig and T. Suzuki on the Martensite Transformation); others are severely practical, addressed to specific industrial objectives (A. G. Chynoweth on Fiber Lightguides for Optical Communications; M. C. Flemings and others on Rheocasting; J. A. Manson on Modification of Concretes with Polymers). Most cover their subjects in breadth, some (N. Brown on The Effects of Gaseous Environments on Polymers; Flemings on Rheocasting) describe only their own work and the problems uncovered thereby, justifiably since they opened up new fields. One particularly illuminating survey, V. F. Zackay's on Thermomechanical Processing, is not so much concerned with gaps in scientific understanding as with the problems of finding a development strategy which will increase the industrial acceptability of the complex form of processing with which it deals. Altogether, the volume is an intriguing study in styles. In general, the broader the author's range of vision, the clearer his presentation. One might have expected that when subject to such severe constraints on the number of words, the narrow specialist would have fared better, but indeed the general principle remains true that one sees the wood better from a helicopter than from the jungle trail.

The Editor does disclaim any attempt to cover the whole spectrum of materials science and engineering, but it is nevertheless true that coverage is lopsided. Nearly 80% of the reviews deal with metals and alloys, and within that category, there is heavy emphasis on the admittedly crucial topic of phase transformations.

It is nevertheless disappointing that only two essays deal with polymers (and one of these only does so tangentially), one with ceramics, and one with oxide glasses (again tangentially). There is no attempt to shirk topics in which understanding is unsatisfactory (E. Rabinowicz has plenty of ignorance to report on Wear, and does so without obfuscation), but no broad topic such as Materials in Biomedical Engineering is included.

In spite of the inevitable limitations and the uneven quality of the reviews, this volume deserves to be widely purchased by junior research workers particularly (the price is set low to enable them to do so). They should use it in conjunction with another compendium of useful short reviews, *Annual Reviews of Materials Science*, to gain a first-class overview of the direction in which the discipline is currently heading. □

A hundred years ago

A VERY extensive Etruscan necropolis has been discovered at Montelparo, near Ascoli-Piceno (Umbria). An enormous quantity of bronze, iron, and terra-cotta objects have been and are being found in the grounds, chiefly consisting of helmets, armillas, collars, buckles, nails, spurs, bows, rings, lances, spears, swords, and thousands of perforated bronze grains and beads, besides numerous objects of amber, glass, shells, and pottery, all of which are likely to be secured by the Italian Government for the Florentine Museums.

Two Prussian officers have arrived in Paris for the purpose of determining telegraphically the longitude of Berlin. Two French officers have been despatched to Berlin in order to carry out similar operations. The apparatus to be used in Paris have been located at Montsouris under the superintendence of M. Mouchez and the Bureau des Longitudes. The ultimate aim of the operation is to connect the French trigonometrical triangulation with the system of the Geodesical International Association which is covering almost the whole of Europe.

From *Nature* **15**, 26 April, 560; 1877.

Book Review Supplement

Advising a President

Edward E. David, Jr

A Scientist at the White House: The Private Diary of President Eisenhower's Special Assistant for Science and Technology. By G. C. Kistiakowsky. Pp. xvii+448. (Harvard University: Cambridge, Massachusetts and London, 1977.) £11.25.

IT HAS BEEN said that history is not made, it is written. It is written after the fact from fragmentary and confused memories, from interpretation of hearsay by people usually not involved, or by using indirect evidence to buttress a preconceived theory. This book is not such a perversion. It has the ring of authenticity, written principally on the spot and at the time by a man involved directly in the affairs reported. From my own experience in the White House with similar situations, it communicates a true sense of the dynamics, the conflicts, the frustrations, the personal friendships and animosities, yet the euphoria and satisfactions of advising a President on matters where he is inexpert.

Dr Kistiakowsky has done a service by publishing his diary. As a diary, it focuses on events, not the process of science advising. As in a diary, specific subjects are fragmented temporally. For example, pieces of the story of the struggle for a nuclear test ban appear throughout the text. There has been no attempt to consolidate these except in brief comments at the beginning and end of the book. This is all to the good, in my opinion, for it lends reality to the account. Yet important issues themselves, such as machinations towards a civilian space programme, can be studied and documented. Scholars of events of that time will find the book indispensable.

For those interested in the process of science advising, the book will be a wellspring of information, even though the subject is hardly addressed directly. Indeed, to the perceptive reader, the process of advising (and the inevitable striving for influence) becomes starkly clear. The flow of events and the colliding opinions and egos document the most critical factor in science advising—knowing the President's deeply-held convictions and values (for example, as outlined above), and relying on them in advocating policy. Convictions and values of a President can sometimes be turned slightly by fact and persuasive argument, but not bent very far.

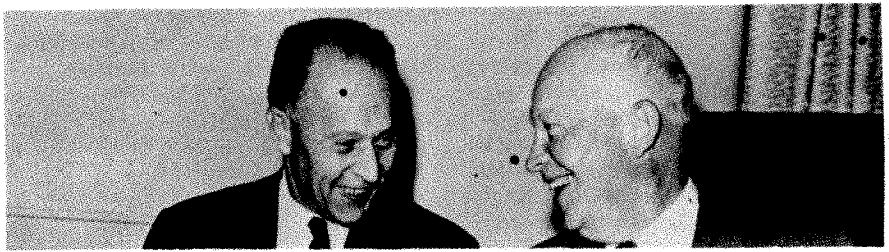
Also fascinating are the personal glimpses of President Eisenhower and his strong commitment to steps for peace. As a military man, he knew the fallibilities of the military, *and* their civilian bosses. He was well aware, too, that budgetary conservatism must be leavened with values which look to the long-term future. He was, therefore, favourable to science and respected the judgement of the professional people involved. The opinions and *modus operandi* of a variety of prominent people both in and out of government are described and judged by Dr Kistiakowsky. Many of them may have rejoinders, if they are still alive and interested. A bit more about that later.

The President's Science Advisory Committee (PSAC) emerge from the diary as a useful instrument, if somewhat obstreperous and self-possessed. The PSAC seemed to function best when it addressed topics at the forward edge of science of technology, but with modest immediate impact on budget, political, or social matters. The PSAC had its detractors on the political side in Dr Kistiakowsky's day as well as in mine. In later times, it became necessary to institute another mechanism—*ad hoc* panels reporting directly to the Science Adviser rather than through the PSAC.

The diary contains a number of significant truths, such as, "This alerted me again to the danger that a 'cold-blooded' scientist's opinion may be very poorly received by the public at large", referring to a PSAC panel's view on carcinogens in food. A larger, still vital truth emerges—that the government requires advice and counsel from knowledgeable private citizens at the top of their careers if it is to act wisely. It must create conditions in which both advisers and advisees are willing.

For me, the book brought back memories and stimulated reconsideration of my own experience in the White House. Perhaps most prominently, I am impressed that events have a startling continuity. Here, 17 years after the events described, many of the same issues are still before us. Only some of the players are different. For every topic that occupied Dr Kistiakowsky, I can recall a derivative topic that occupied me some 12 years later. He expended effort on the nuclear test ban, I on an extension of the ban and on SALT I. Principal players in his time were John McCone and Christian Herter; in my time, they were Henry Kissinger and David Packard. Dr Kistiakowsky was concerned about large space boosters, I about the space shuttle. He dealt with Keith Glennan and Herbert York, I with James Fletcher, George Low and Donald Rice (then OMB, now President, Rand Corporation). Dr Kistiakowsky worked on the carcinogenic content of cranberries and the rigid Delaney Amendment, I on the pesticide 2,4,5T and cyclamates (today, saccharin). His correspondent was Arthur Fleming, mine were Elliot Richardson and Charles Edwards. Dr Kistiakowsky tried to put forward an acceptable high energy physics programme particularly advocating the Stanford linear accelerator; I did likewise trying to keep accelerator facilities from budget-induced idleness, and I also advocated heavy-ion research and radio astronomy, including the X-ray spectrum. He dealt with Maurice Stans, I with George Shultz and Caspar Weinberger. Dr Kistiakowsky addressed the strength of Western *vis-à-vis* Soviet science, and that was a hot topic in my time too. Dr Kistiakowsky's time saw the demise of the U-2 and its surveillance of the Soviet Union. He also saw in the SAMOS programme—the beginnings of satellite surveillance as a replacement. This topic remained an important area for the science adviser until the end of my own term. The U-2 incident which 'spoiled' Eisenhower's tenure has led to a new era in which 'national technical means' is a technique accepted tacitly by both sides as insurance against military surprise. Thus, today's intelligence estimates can speak confidently about the size and character of Soviet miltary deployments and developments, even though the intent behind them is and will remain a subject of dispute.

But there were also new concerns in the early 1970s. Energy was a principal one; including the breeder reactor programme; so was health care, as distinct from biomedical research. (There was a concern in both areas about the performance and *modus operandi* of the NIH, a concern that persists at least in the political arena to this day.) Directed biomedical research, typified by the Cancer Crusade, became an unwise *cause célèbre* in the 1970s, beginning in the late 1960s under President Johnson. In my time, there was a social science focus on the adolescent maturation process. That concern was stimulated by the perceived decline in educational quality. There was increasing attention to social science research programmes and modelling as an aid to understanding their workings (including the Limits-to-Growth convulsion). And above all, there was the concern about environmental pollution and the drive to control it. A central issue in my time was the pollution caused by automotive and coal-burning plants.



Kistiakowsky (left) with Eisenhower

UPI Telephoto

Some of these 'new' subjects had begun to surface in Dr Kistiakowsky's time. But like an older person whose eccentricities have become exaggerated, the Science Adviser's office, as it matured in the 1970s, became more and more concerned with these 'offbeat' topics, which ranged far beyond the earlier principal concern—the physical sciences. The roots of this trend are clear in Dr Kistiakowsky's diary. Some say this was the downfall of the office, but it wasn't. It was a necessary evolution to keep pace with presidential interests.

Beyond both latent continuity and its contrast with future events, the diary indicates clearly Dr Kistiakowsky's incisive judgments about issues and people, some of the latter not very complimentary. Undoubtedly, those people would have retorts to Dr Kistiakowsky's judgment of them. Furthermore, events are described in the diary as perceived by Dr Kistiakowsky at the time. It is remininscent of the famous Japanese film, *Roshamon.* Its lesson was that each participant in a happening emerges with his own perceptions, very different from those of the others. Knowing that lesson, I took the liberty of talking with some of the other participants in a few of the events described in the diary. Indeed, at least two of those people had entirely opposite perceptions of the same events from Dr Kistiakowsky's, based not only on their memories but on their own diaries. Clearly, there has been no attempt to deceive the reader in Dr Kistiatkowsy's diary; rather, at work are the usual divergences of perceptions conditioned by participants' past experiences and personal egos, and the resulting preconceptions. Thus, the reader should realise that there are other sides to the stories as told in Dr Kistiatkowsky's diary. A tell-tale sign is that Dr Kistiakowsky saw his judgments in the complex matters he described as almost infallibly correct.

Significantly, Richard Nixon as Vice-President appears several times in the book. The text of the diary may leave the impression that he was often on the 'right side' of the argument, and so somewhat enlightened. Yet, there is a second impression which emerges on careful reading—that Nixon knew how to judge an occasion and take an acceptable position. Dr Kistiakowsky, in his notes added last year to his diary, says he came into the White House with a negative attitude towards Nixon, found him self-centred, and "remained sceptical to the end of my stay in Washington."

This book is the most significant account of the first era of Science Advisers yet published. The post was established in 1957 and abolished in 1973, about six months after I left the White House. It was re-established by an Act of Congress, signed by President Ford in May 1976. The Carter Administration's appointee is Dr Frank Press, an eminent and capable Earth scientist. With his installation begins a new era of presidential science advice. I hope that during its span, even greater accomplishments will be added to those of the first era—a significant piece of which is recorded in Dr Kistiakowsky's book.

An accolade is due to Professor Charles Maier who encouraged the publication and added a highly useful introduction to set the stage for the otherwise cryptic entries which follow.

Edward E. David, Jr, was Science Advisor to President Nixon and Director of the Office of Science and Technology (Executive Office of the President) from 1970 to 1973. He is now a consultant, based in Chicago.

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Experimenta lucifera

Erwin Chargaff

The Professor, The Institute and DNA. By R. J. Dubos. Pp. 238. (Rockefeller University: New York, 1976.) $14.50.

In the late 1930s, during my early days at Columbia University, I had frequent occasion to visit the Rockefeller Institute for Medical Research, as it was then called. The uninviting structures on Avenue A near the East River were not easy of approach. True to its character of a citadel of learning, the door of the Institute was fortified by gruffness; and after passing inspection by Cerberus or Cerbera, the visitor had to be accompanied to his destination by a special young man. In my case this destination usually was the laboratory of the organic chemist Max Bergmann, much admired by me. I had known Bergmann when he directed a Kaiser Wilhelm Institute in Dresden; here in New York he shared the uneasy fate of his generation of German emigrants. On seeing me, he would exclaim: "*Zuerst rauchen wir eine Friedenspfeife!*" producing a large glass jar in which a package of cigarettes was kept in a controlled habitat. Occasionally, he conducted me afterwards to another laboratory, that of P. A. Levene or D. D. van Slyke; and sometimes I would come upon the light-brown shadow of an elderly mouse-like figure tripping along the corridor walls. This, I was told, was Dr Avery, a name not unknown to me as that of the greatest expert on the pneumococcus, although at that time I could not have known how important his name would become to me a few years later.

These reminiscences were brought on by an uncommon book about an unusual man, Oswald T. Avery (1877–1955). It is, in my opinion, a very good book, and I enjoyed reading it. As the title indicates, this is not merely a scientific biography. The book operates on several intercommunicating levels, taking into account the man, the time, and the place; and painting, with extraordinary competence, the ever-changing human and scientific backdrops. This competence is not entirely surprising: René Dubos, apart from being a very good writer, was a member of Avery's department from 1927 to 1941, and the warmth of personal contact and observation is felt throughout the narrative.

Born to English parents in Halifax, Nova Scotia, Avery was taken to New York City at the age of ten, when his father, who was a Baptist clergyman, was invited to be pastor of the Mariners' Temple on the lower Eastside, even at that time a pretty horrible part of the city. But five years later, the father was dead, and the three sons were brought up by the mother who must have been an energetic lady. Oswald Avery, aside from becoming an accomplished cornetist, got a good education: first something called, soberly, the New York Male Grammar School, then Colgate Academy, Colgate University, and finally, between 1900 and 1904, the College of Physicians and Surgeons of Columbia University, which was then one of the best finishing schools for clinicians. The latter seems to have finished him in more than one sense: in went a lively, communicative young man, majoring in the humanities, excelling in oratorical contests, playing the cornet, leading the Colgate University band, and clearly not particularly attracted to the natural sciences; out came what I would term, perhaps with some exaggeration, a scientific recluse. This shock of confrontation is not a very rare event: I have noticed it often in several generations of medical students, when I taught at the same medical school; only few of those affected, I am sure, turned into other Averys; mostly they became psychiatrists or even psychoanalysts.

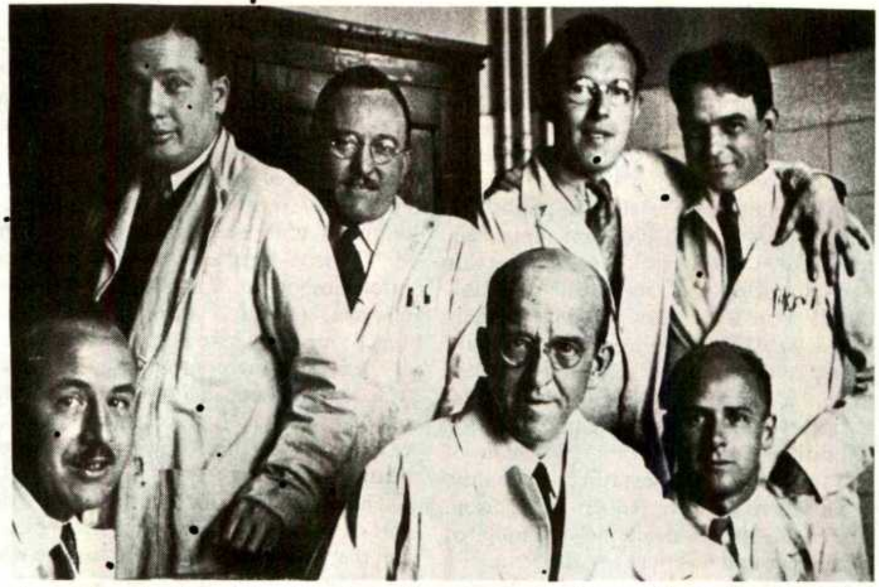
Scientists in general lead uneventful lives, with the exception of the few who, for instance, are guillotined during the French Revolution or killed by highway robbers in Southern France. What counts is their inner history to which their published papers afford only precarious access, for the history of ideas, and especially of scientific ideas, is a slippery discipline. But in the present instance, Dubos has succeeded in producing as multidimensional an image as is possible.

With the exception of a very short period, after graduation, in medical practice, Avery devoted his entire life to research in bacteriology and immunology, first in a now extinct private institution, the Hoagland Laboratory in Brooklyn, and since 1913 in the Rockefeller Institute, where he remained until he was 71. The last few years of his life were spent, let us hope serenely, in the South. Or as Dubos puts it: "In 1948, he decided that he had shot his bolt; as he no longer felt able to function effectively in the scientific arena, he retired to Nashville, Tennessee". Each of the verbs in the preceding sentence could lend itself to a philosophical analysis which I shall not attempt here, except to wonder why society seems to reserve circus acrobatics and science for the very young, assuming that these occupations require muscular vigour rather than wisdom. Why had, for instance, Telemann not yet "'shot his bolt," when at the age of 81 he wrote what is widely considered as his greatest work, the oratorio *Der Tag des Gerichts*? The multiplicity of possible answers shows that we have not yet found the corect one.

The major part of the book is devoted to a detailed and lucid discussion of the problems investigated by Avery during his 35 years at the Rockefeller Institute. This is done in five chapters the titles of which will indicate the areas under study: 'The Lure of Antiblastic Immunity and the Chemistry of the Host'; 'The Chemical Basis of Biological Specificity'; 'The Complexities of Virulence'; 'Bacterial Variability'; and 'Heredity and DNA.' It will be recognised that Avery was one of the early microbiologists who understood the dominating role that chemistry was to play in biology. This was, incidentally, quite in harmony with the *genius loci* of the Rockefeller Institute, which in this respect, as in many others, was a most remarkable place. Dubos does full justice also to this side of his story; and the Institute, with its members, seminars, conferences, and, especially, its memorable capitalistico–monastic lunch room, is one of the indispensable elements of his account.

Avery was comparatively late in starting, but he lasted: his most important work was published when he was past sixty-six. The pneumococcus was his microcosm; he showed that general principles of great import can be derived from little things if it is given to the researcher to join penetration to perseverance, and bold deduction to honest induction. As always, what counts is the balance, the mixture; but has anybody in science succeeded in mixing himself, in filling his own recipe?

Avery became interested in the pneumococcus because one of the principal projects studied at the Rockefeller Hospital was the development of a serum therapy for lobar pneumonia. I do not believe the practical results of his research ought to be stressed, but out of this work there emerged a new understanding of the chemical basis of antigenicity, and, even more suprisingly, the recognition that genes were made of DNA. These glories may be taken to demonstrate the stupidity of our era of target-directed research. Actually, science has never operated entirely without goals; but the goals were chosen by a few reasonable men, not by frightened politicians or bureaucrats, and were enforced with



Avery's Department, 1932.
Seated (left to right): T. Francis, Jr, Avery, W. F. Goebel

Standing: E. E. Terrell, K. Goodner, Dubos, F. H. Babers

tact and imagination. The directors of the Institute and the Hospital were wise enough to leave such a man as Avery in peace. They had trust in him; something that no 'peer group' with its silly priorities can afford or accomplish. The absence of frenzy is one of the main impressions I get from Dubos' description of Avery's laboratory.

The existence of many immunologically different pneumococcus types had been known for some time. In 1916, Avery's intimate friend, Alphonse Dochez—I knew him very well during his years as a Columbia professor—discovered that type-specific soluble material was released into the culture fluids by the organisms. These observations, extended and refined in the course of several years, finally led to Avery's collaboration with Michael Heidelberger, and later also with Walther Goebel, and to the identification of a host of type-specific bacterial polysaccharides as the basis of the immunological specificity of the various strains. It is not too much to say that this work had a profound influence on the growth of immunochemistry and on later concepts of "the chemical aspects of biological specificity." This was the title of the series of Jesup lectures that I gave at Columbia University four years after Avery's death. His name was mentioned more often than anybody else's, with the exception, of course, of my own.

Leaving aside a large number of interesting and important investigations by Avery and his collaborators—almost all within the confines of his 'pneumocosm'—I should like to move rapidly to what most of us will consider the most illuminating, the *luciferrimum*, of his many *experimenta lucifera*. (Dubos quotes Francis Bacon's distinction, in his *Instauratio Magna*, between "experiments of light" and "experiments of fruit.") I refer, of course, to the work on the biological activity of DNA. In view of the witches' brew now being stirred all over the world, with "recombinant DNA" as its main ingredient, I can only hope that the title of this essay will not have to be changed to *experimenta Luciferi;* although the Devil hardly needs experiments to make his point.

Less than sixteen years—1928 to 1944—were required for the first fundamental observation to lead to the definite proof that DNA was the instrument of genetic specificity. That it actually took much more time before this proof was accepted generally, was due to obtuseness, malevolence, and the desire to protect various vested interests. I remember the names of both the heroes and the villains in this story, but only a few of the first will be mentioned here. When the transformation of pneumococcal types *in vivo* was discovered in 1928 by F. Griffith, there were no loud objections, perhaps owing to the rapid confirmation of his findings in other laboratories or because most bacteriologists at that time were Lamarckians. But for some reason the observations were filed away and, had it not been for Avery, they might have slumbered a long time. It was in Avery's laboratory that Dawson and Sia achieved transformation in *vitro* and that Alloway described the isolation of a crude transforming factor. All this was accomplished before 1933; and Dubos takes great pains to explain why more than ten years elapsed before the next, and in every respect final, publication. Such explanations are really not necessary: before World War II, science was not yet an achievement sport; speed records formed no part of the accomplishments of a scientist as they do now. Griffith and Avery are both quoted as deprecating hurry; and Dubos tells us that Avery liked to recall "the words of an old black patient who watched, with amused surprise, the young doctors rushing about the wards of The Johns Hopkins Hospital: 'What's your hurry, Doc? By rushing that way, you passes by much more than you catches up with!'".

When in 1944 the epochal paper by Avery together with Colin M. MacLeod and Maclyn McCarty appeared, it certainly was something worth waiting for. The stages leading up to this publication and the all-in-all shabby reception granted it by the experts are well documented in the book, although more could, and probably will, be said eventually. As to the effect that the identification of the transforming principle as a form of DNA had on me and on the direction of my subsequent work, I have described it before. Avery himself obviously realised the implications of his discovery much more profoundly than he was willing to put into print. The letter he wrote to his brother Roy on 26 May, 1943, is particularly instructive in this respect. His entire character as a scientist—relentless perseverance, courageous imagination, extreme caution—can be developed from this document.

Many readers will, I am sure, find one chapter especially moving—namely, chapter twelve, entitled "As I Remember Him." I have never seen this done before in the biography of a scientist. To the limited extent that a scientific investigator is also a human being, the carving of the private bust calls for an unusually tactful and sensitive observer; and this René Dubos must have been in the many years he spent with or near Avery. Nevertheless, Avery was an extremely private person, and there must have been a wall around him, not of his own building—a wall that constrained him as it restrained access to him. I am not sure that we can ever understand another man so as to resurrect him on paper. The reason why figures invented by great novelists strike us as so alive is that they are invented. At any rate, what I got out of reading about this shy, puritanical, disciplined, and cautious man was a renewed awareness of the poverty of greatness.

As I said at the beginning, this is an interesting book. It is also very well produced, with 22 illustrations, some quite fascinating, and with a good index. It should be read by all who consider themselves part of the 'biomedical community', and molecular biologists should read it twice. Even philosophers and historians of science, if they can spare a few moments from their contemplation of the dark side of the Reverend Moon, will find the book profitable. ☐

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Conversations with Haldane

Eric Ashby

The Man with Two Memories. By J. B. S. Haldane. Pp. 220. (Merlin: London, 1976.) £2.80.

THE style is unmistakable; it has the taut logic of Handel's music. So is the puckish barbed humour: "As I was at a day school, I did not adopt homosexual practices . . . ". And so are the sparks of exotic knowledge: "Of course I have a fair repertory of languages, including one or two unusual ones, such as Hittite, which I had to learn in order to study the earliest serious biological document, Kikkalish's instructions for training chariot horses." No-one but J. B. S. Haldane could write such stuff in such an enchanting style. Those who recollect his brilliant and sometimes exasperating conversation will find in this book a nostalgic re-creation of what it was like to spend an evening in his company. Those for whom J. B. S. Haldane is a somewhat romantic myth—a man who could turn from mathematical genetics to polemics on sociology in the *Daily Worker*, and who used to leave his laboratory at the end of term to spend the vacation in the civil war against Franco—will get an impression of what it was like to be a contemporary of the man.

I remember him at a dinner of the Society for Experimental Biology. It was a light-hearted informal occasion and for some reason J.B.S. was asked to make a speech. In that grave, hesitating voice, as though about to begin a scientific lecture, he said that although he could not bring himself to believe in heaven, he now had some inkling of what heaven would be like if there were such a place. For he had greatly enjoyed this dinner, "thanks to the delectable company of the young lady on my right and the young lady on my left. Now in a three dimensional world one can have only two ladies sitting next to one. Heaven I believe, might be conceived to be, a place in *n*-dimensional space, where one could therefore expect at dinner to enjoy the company of *n*—1 young ladies."

This book is a mixture of J. B. S. Haldane's startling breadth of knowledge with his sparkling and often grotesque imagination. It is a fantasia with a purpose. The purpose is to expose by ridicule the prudery and bigotry and credulity of twentieth century western civilisation and to put on record glimpses of the sort of utopia he himself would have enjoyed. It is a work published years after his death. The blurb on the dust cover suggests that it may be regarded as a philosophical and scientific testament. Anyone who opens it with this expectation will be disappointed. It has neither the cohesion or the conviction of a testament. It is a jumble of ideas, leaps of imagination, and exhibitions of prejudice which I don't think Haldane himself would have wished to publish. It has not got enough plot and movement to make it good science fiction, nor enough austerity of argument (and Haldane's scientific work was impeccably well argued) to make it good scholarship. Nor, I believe, did Haldane himself intend it to be in either of these categories. It is simply a feast comprising the after-dinner conversation of a man of extraordinary and fascinating personality.

The 'hero', James Robert Murchison, carries in his brain all the accumulated experience and response to experience of a British scientist. But there has also entered his brain and lodged there and become manifest to Murchison, all the accumulated experience of Ngok Thleg, a being from another planet. It is a case of memory transfer which can occur if two persons have similar genotypes. The planet from which Ngok's memory-and-experience bank comes has solved many of the scientific and social problems which perplex planet Earth. The people there may multiply by clones; anti-social behaviour is promptly cured by injections of drugs; the unit of time is that taken for light in a vacuum to traverse 2.317×10^{17} wavelengths of the red hydrogen emission line; cities are built of an alloy of titanium and are enclosed from the atmosphere, kept at a 10% higher pressure with pumps and filters to remove bacteria (disease, therefore, was an anachronism). There is some interplanetary travel; some computerised information services; and a little highly aseptic permissiveness: all developments which have made some headway since Haldane's death.

The book is unfinished. It is not the sort of work which readers of *Nature* need feel they *ought* to read. But if anyone who is going by train from, say, London to Edinburgh would like to have J. B. S. Haldane's company for the trip, talking rather as he did to me when I made that journey with him some 40 years ago, this book will give him that experience. ☐

Eric Ashby (Lord Ashby of Brandon) recently retired from the Mastership of Clare College, Cambridge.

Father of ethology

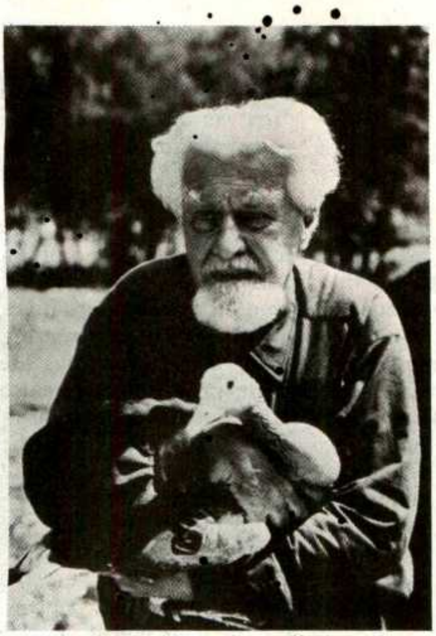
Aubrey Manning

Konrad Lorenz. By A. Nisbett. Pp. xiv+240+21 photographs. (Dent: London, 1977.) £5.95.

BIOGRAPHIES of living people, particularly those around whom controversy has gathered, are risky ventures. It is usually better to let the dust settle so that the subjects are seen more clearly in relation to their own time and place. Konrad Lorenz is clearly such a subject but Alec Nisbett's book is successful in avoiding the main pitfalls: this is a thoughtful and substantial biography. Nisbett has a background in mathematical physics but made a career in the British Broadcasting Corporation, where he has won many awards for his skills in presenting scientific subjects to the general public. His interest in Lorenz arose from such work, and having obtained grant to work on this biography, he spent much time talking to Lorenz and some of his key associates. Nisbett obviously likes Lorenz and so does this reviewer. Consequently for both of us there is the danger of becoming indulgent and less than adequately critical when discussing Lorenz's ideas and influence. This does set out to be a critical biography but the soft pedal is certainly applied in some places.

Konrad was the younger son of Adolf Lorenz, a successful orthopaedic surgeon (he almost got the Nobel prize for medicine himself) who identified with the Austro-Hungarian aristocrats and built himself a large house in the baroque style at Altenberg near Vienna. (Konrad has returned to this same house in retirement.) Adolf wanted his son to follow him into medicine: he did qualify, but already the jackdaws, herons, ducks and geese had taken over his life. Lorenz lectured on animal behaviour in Vienna, but had no formal research facilities; the animals all lived at home. During this period he published all his most influential papers and began collaborating with Tinbergen. Academic recognition was crowned by Lorenz going to the prestigious chair of psychology at Königsberg (then in East Prussia). The war had already begun and soon enveloped the eastern front. Lorenz ended up practising simple medicine and writing on philosophy in a Russian prisoner-of-war camp.

He was not repatriated until 1948 when he finally got back to Altenberg to reconstruct his career. It was much harder to regain academic recognition in Germany. No university position was forthcoming although a host of new students were gathering around Lorenz. He worked more or less on an *ad hoc* basis until the Max Planck Institute enabled him to obtain facilities at Seewiesen in 1955. He retired back to Altenberg with a smaller research institute in 1973.



Lorenz tending a wounded goose

Nisbett provides an excellent account of Lorenz's life and skilfully manages to interweave comments on his scientific thinking. I had some contact with the developments in the mid-1950s, and he very successfully catches the atmosphere of the post-War emergence of ethology in Europe. There can be no doubt of Lorenz's key role in its birth. He *is* the father of ethology, however many of his children now reject his ideas.

The early papers in which Lorenz developed the concepts of the fixed action pattern, the releaser and the evolution of displays, of action-specific energy and of imprinting, are all the result of dedicated observation and description shot through with brilliant intuitive thinking. The approach to the animal in its natural environment and the concern with function and evolution came as a powerful tonic to behavioural science stultified by white rat experimental psychology. To read Lorenz's papers was enough, but to hear him lecture—the flood of words and ideas enlivened by his repeated assumption of diverse animal forms on the platform—was to experience instant conversion. Ethology took off, and although Lorenz himself is quick to point out his debts to Whitman, Craig, Huxley and Heinroth, it was he himself who founded the school.

Lorenz's own great period was over by 1960. He had little taste for the quantitative and analytical studies which had to come next—he boasts that no paper of his ever included a graph; nor can I recall any tables. The new impetus was provided by Tinbergen, who rapidly built up a group in Britain, and by groups in the USA.

Lorenz turned increasingly to the application of his original ethological concepts to human behaviour and his weaknesses began to overlie his strengths. He had always tended to adopt rigid classifications in behaviour: instinct or learning, genes or environment. His disputes with the "English-speaking ethologists" as he referred to them, largely centred on their refusal to accept such dichotomies. Lorenz failed to appreciate that they were just as interested in the genetic basis of behaviour as he was, but often chose to approach the question through the study of development. It no longer remained helpful in such research to classify elements of behaviour according to any fixed scheme. When Lorenz began writing on the inherited basis of human behaviour he showed few signs of compromise. With the publication in English of *On Aggression* in 1966, the storm broke; Lorenz and those who supported his deterministic views on human behavioural development were ranged against the cultural and environmentalist schools.

As always in nature–nurture controversies, politics were to the fore. Lorenz's alleged Nazi sympathies during his Königsberg period were focussed on by the environmentalist left, especially when his Nobel Prize was announced. Nisbett provides a good account of the particular paper which gave such offence. He points out how certain mistranslations have been at the base of misunderstanding and misrepresentations. What Nisbett doesn't do adequately is to point out that whether or not Lorenz's views on the dire effects of domestication in man (whatever that means) were dressed up for Nazi consumption, they were in any case remarkably bad science.

Lorenz's reactions to the Nobel Prize furore saddened many of his friends. He admits himself that once on camera he becomes a buffoon. Instead of dismissing a youthful error at once, he tended to become defensive. He certainly gave a totally misleading impression at times. Nobody who knows Lorenz personally can doubt his warm humanity.

Nisbett's book will serve as an interim statement as it were. He gives a most useful list of references both to Lorenz's own work and to other writings which bear on his ideas. Perhaps the centre of gravity of this biography is inevitably towards the later period of Lorenz's career. Nisbett rightly feels that he must discuss Lorenz in relation to the controversies of recent years. When we come to look back at Lorenz's contribution in more tranquility, however, it is *King Solomon's Ring* that will stand out as his great book. Within its easy charm are all the important ideas which make up Lorenz's profound contribution to the study of behaviour. ☐

Aubrey Manning is Professor of Natural History at the University of Edinburgh, UK.

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Richard of Wallingford

Richard Lorch

Richard of Wallingford: An Edition of His Writings, with Introduction, English Translation and Commentary. Edited by John D. North. Three Volumes: pp. 1,312; 22 plates; 128 text figures. (Oxford University: Oxford and London, 1976.) £75.

RICHARD OF WALLINGFORD (about 1292–1336) was among the ablest astronomers and instrument-designers of the Middle Ages. His *Quadripartitum*, for all its prolixity, bears comparison with Regiomontanus' *De Triangulis*, written a century and a half later; and his astronomical clock was one of the most advanced made in the fourteenth century. Again, the complex universal instrument 'Albion' ('All-by-one') was a remarkable achievement, and was fully appreciated by fifteenth-century astronomers such as John of Gmunden (about 1382–1442) and Regiomontanus (Johann Müller of Königsberg, 1436–76), who, among others, re-edited and commented on the *Tractatus Albionis*, in which Richard describes the instrument. Dr North has provided us with a reliable and annotated edition of all Richard's works. From this monumental edition, which must surely be ranked with Nallino's edition of Albategni, we may gain insight not only into Richard's achievements in astronomy, astrology, the making of instruments, and mathematics, but also into the progress of these sciences throughout the Middle Ages.

In studying any western astronomer of the high Middle Ages it is necessary to pay attention to borrowings from his predecessors, particularly the Muslim astronomers whose works were translated into Latin in the twelfth and thirteenth centuries. On the whole, Richard of Wallingford is explicit about his sources, but some problems remain. Thus, in the first part of the *Tractatus Albionis* he gives chapter and verse for each proposition, the usual sources being—besides Euclid and Ptolemy—Albategni's (died 929) *Opus Astronomicum* and an "abreviated Almagest". As is pointed out in the commentary, the latter is better known as the *Parvum Almagestum*. This is probably a Latin compilation and has been attributed (by A. Birkenmajer, *Studia Copernicana* I, 143–6) to one Walter of Lille, although Campanus of Novara (died 1296) is also a possibility. There are at least two variants of the *Parvum Almagestum*, quite apart from variations in the mainstream text, and one of these could well account for the remaining references to a "Commentator" on the *Almagest*. At all events, this Commentator is unlikely to be Averroës (1126–1198), whose commentary on the *Almagest* was apparently unknown in Latin (despite Birkenmajer's citation of the fourteenth-century Alphonse Dionysii de Lisbonne, who probably thought the *Parvum Almagestum* was by Averroës); and was so differently organised as to make the identification unlikely.

The sources of the *Quadripartitum* are not given with such readiness, but Dr North has discovered the most important of them: a text beginning "*Proporcio est duarum quantitatum* . . . ", which he for several reasons ascribes to Campanus. There are some valuable remarks in the commentary about the whole history of "cata" (Menelaus' theorem) and of ratios: it is to be hoped that these notes and the authors cited in them will encourage someone to make a critical edition of the main texts. The *De Sectore*, a reworking—apparently by Richard himself—of the *Quadripartitum*, is not printed in full. It is of some interest since it incorporates the new trigonometrical methods developed by the Muslim astronomers and introduced into the West by the translation of the astronomy of Geber (flourished during first half of twelfth century). Whether later English writers like Simon Bredon (about 1300–about 1372) copied Geber's methods direct or through the *De Sectore* is a matter for further research. What we do know is the approximate date of their introduction into England.

Richard of Wallingford (Cotton MS Nero D VII, f20)

Courtesy British Library Board

Of the shorter works the treatise on the "rectangulus" is perhaps the most interesting. Characterised by Henri Michel as "un véritable *Torquetum* schématisé", the rectangulus was invented by Richard to "obviate the tedious and difficult work of making an armillary sphere". (Anyone who has made even a rough model of an armillary sphere will sympathise.) Dr North points out that such alternatives cannot be lightly dismissed as observing instruments, and further maintains the unfashionable view that they were also used for the transformation of coordinates. It is interesting that in both the *Rectangulus* and the *Albion* Richard follows the custom, found among Muslim writers, of listing the parts of the instrument, together with their technical names. Franco's treatise on the "torquetum" is a possible intermediary.

Some of the texts included in this edition—for instance, the canons to John Maudith's tables, with which volume 1 begins—are here attributed to Richard Wallingford for the first time; and the authorship of doubtful cases, such as the astrological tract *Exafrenon*, is fully discussed. The account of Richard's life, besides being interesting in itself, contains some useful remarks on biographical sources for Englishmen of this period. The texts themselves are mostly established from two or three good manuscripts (in some cases discovered by Dr North), the others being used in a subsidiary role. There are occasional omissions—no doubt following Bjørnbo's edition of Thabit's *De figura sectore*—of purely repetitive matter, but synopses are given to fill the gap. On the whole, the English translation, on facing pages, is literal; but, where appropriate, the material is clarified by the use of modern symbols and line-by-line layout of the argument. As one would expect from the author of *Kalenderes Enlumyned Ben They*, the Middle English translation of the *Exafrenon* is given in preference to a modern one.

The three volumes are conveniently arranged so that the text and translation, commentary, and diagrams, can all be seen at once. In volume 3 there are plates, indexes, glossaries, and no fewer than 39 appendices. Some of these appendices and some of the longer disquisitions in the commentary volume are complete in themselves, and form a collection of well-indexed articles on the periphery of the central theme. We can only hope that their author is not preparing to abandon the Middle Ages. ☐

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Newton as alchemist

A. Rupert Hall

The Foundations of Newton's Alchemy. By B. J. T. Dobbs Pp. xv+300. (Cambridge University; Cambridge, 1976.) £10.50.

EVER since David Brewster published his Victorian biography of Isaac Newton 120 years ago it has been known that Newton devoted much time to chemical experiments and alchemical authors; indeed, Brewster, while faithfully noting his hero's purchases of chemicals, apparatus and Ashmole's *Theatrum Chemicum,* lamented his waste of time on Ripley, Flamel, Starkey and the rest. Moreover, Newton's interest in these matters has long been evident from his correspondence. The dispersal of the great mass of Newton's private papers in 1936 simply made openly available a mass of material that had been formerly known but largely inaccessible, and which in any case had not been regarded as very important to those who were more interested in Newton as the founder of mathematical physics than as a man of his own age.

The mood has changed, so that during the past thirty years many have pursued Newton the whole man, rather than the author of *Principia, Opticks* and some mathematical treatises. 'Freudian' analysis has laid bare the mechanics of his personality, and he has been portrayed as theologian, historian, Mint Master, magus, and most recently as alchemist. How much light all this knowledge derived from deep reading and wide scholarship actually throws on Newton's role in the history of mechanics or optics is still an undecided question. The first attempt, by David Castillejo, to discern a unity in all Newton's work has remained unprinted (although copies are on deposit in certain libraries). In her new study of the earlier phase of Newton's interest in chemical topics Mrs Dobbs, after setting out the background that Newton inherited, investigates the kind of information about the nature of metals that Newton was looking for in his alchemical authors, and relates this to his own experimental endeavours. Finally, like Mr Castillejo (but more precisely), she explores the relations between alchemy, theory of matter and the concept of gravitation.

There is a difficulty in this aspect of Newton's work—for it was certainly a serious enough activity to be judged work—which is encountered in studying Robert Boyle and others too. Newton actually published in his lifetime certain perfectly rational views about chemical properties and the nature of matter (which were not without influence on the development of chemistry as a science). We also possess factual and fairly straightforward accounts of experiments that he made on such and such days written in his own hand. We have again personal and authentic expressions of interest as in his letters to Aston (as early as 1669) and to Locke about Boyle's supposed incalescence of gold with mercury (August, 1692). And in his London years, later still, Newton kept up an acquaintance with the alchemist William Yarworth. Finally, there is the known extent of his own alchemical library and of his voluminous extracts from alchemical authors, by no means excluding those of the esoteric sort. What is largely (but not, it seems, absolutely) absent is any comment, explanation, or reworking of this last material from Newton's own pen. His purposes, his intentions, his possible ambitions in collecting and studying it, have to be for the most part inferred.

As is evident from Newton's published chemistry, and from the experimental records first analysed by my wife and myself many years ago, Newton's mind in all this worked at a rational, factual, indeed quantitative, level. In this respect Newton's pyrotechnical work shows no difference of principle from his work in optics. If this were not true it is impossible to understand how *Query 31* or *De natura acidorum* could have been written. Much as we proposed nearly twenty years ago, Mrs Dobbs writes of Newton's translating alchemical processes into chemical terms which he could then subject to trial in the laboratory. If he plunged for enlightenment into the "most esoteric and mysterious productions of the alchemists", he sought their meaning by 'rational analysis', and experimented methodically, almost ploddingly (pp168 and 175).

Illustration shows the 'greene lyon' devouring vivifying celestial influences, symbolised by the Sun, and emitting a vivified mercury, the living or 'actuated' character of which is symbolised by blood.

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In Boyle's and Newton's day it was of course perfectly rational to suppose that metals might be compound bodies and capable of chemical modification. According to Mrs Dobbs, in a manuscript of his own composition headed *Clavis* (Key), Newton wrote out a detailed account of a process for preparing "a mercury dissolving all metals, particularly gold". (Its humourless author noted that "one philosophical sign will appear to you: in the very making of the mercury there is a great stink".) Such solution was preliminary to the complete mastery of metals. Like Boyle, the writer of the *Clavis* saw gold apparently fermenting and changing colour in this menstruum.

What is rather at stake is the question of Newton's commitment to the alchemists' ideas. Did their writings not merely contain hidden clues to actual procedures which could be verified or falsified in the fire, but a profound esoteric insight into the workings of nature? Here (as also in Mrs Dobbs' conjectural "school" of alchemy in Cambridge, whose leading activists were Isaac Barrow and Henry More, with Newton as their neophyte) it is less easy to accord with her views. It is true that Newton in *Opticks* and in the discussion of comets in the *Principia* hints at the transformation of a cosmic 'spirit' into water and so (as Boyle thought he had proved) into earth; such a progressive coalescence of the fundamental particles of matter (neither fermions nor bosons!) was not forbidden by contemporary theory, and had indeed been invoked by Descartes.

There are other ways too in which, as Mrs Dobbs rightly points out, Newton's faith in the "analogy of nature" led him into strange, or at least empirically unjustified speculations. But it seems needless to derive the concept of gravitational "attraction" (which Newton after all declared did not need to be an attraction and was certainly not inherent in matter) from alchemical origins, and extreme to affirm that "in a sense the whole of his career after 1675 may be seen as one long attempt to integrate alchemy and the mechancial philosophy" (p230). Are the mathematical papers in Dr Whiteside's eight volumes equally to be seen as of no significance in Newton's intellectual development?

Nevertheless, even the reader who may feel some reluctance to substitute *Caput Mortuum* for *Caput Draconis,* and some suspicion of any single key to the secret of Newton's greatness (many have been offered), must find his understanding of Newton broadened and stabilised by Mrs Dobbs' explorations of the more obscure recesses of his mind. ☐

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Scientific classic of history

J. D. North

Copernicus: On the Revolutions of the Heavenly Spheres. Translated by A. M. Duncan. Pp. 328. (David and Charles: Newton Abbot and London; Barnes and Noble/Harper and Row: New York, 1976.) £12.50.

JUST as in the Middle Ages the Bible and Liturgy were encased in Latin for the almost exclusive use of the priesthood, so in our own time most of the scientific classics of history remain either untranslated or—what is worse—badly translated. It should not be necessary to plead the case for a translation of the *De Revolutionibus* by Copernicus. The inadequacies of that prepared by Charles Glenn Wallis, and published in the series *Great Books of the Western World,* are easily exaggerated, but too numerous to make it an acceptable student edition. Dr Duncan's new translation is not only better, but it has the virtue of being separately available—that is, without an obligation to purchase the other 53 volumes of a collection.

Dr Duncan's style is modest, simple, and direct. His introduction could be readily understood by any sixth-former. The notes and introduction are not very long or profound, but neither are they likely to frighten away the inexpert reader. The heliocentric idea is kept to the centre of the commentary, where it belongs, psychologically if not technically. The simplicity of the commentary is, however, occasionally misleading —as when the so-called theory of trepidation is considered, and as when modern disputes over Copernicus' Aristotelianism and the reality of the spheres are deftly swept under the carpet. Copernicus's conservatism in the use of Ptolemaic methods and parameters is stressed, but Copernicus is represented as having made an advance on Ptolemy by the introduction of systematic observational tests of his model. The truth of the claim—simply because it is made so briefly in the introduction—is itself difficult to decide, but there are many who will find traces of special pleading in it, at least as far as it concerns Ptolemy.

The notes to the translation are slight and sparse, and the bibliography at the end even sparser. There are no indexes. The translation occupies almost nine-tenths of the book, and it is of course by this that the volume must primarily be judged. Speaking generally, the version is accurate and readable, and if it were possible to review the translation at pedantic length, most of my own objections would concern matters of taste rather than of substance. For example, out of kindness to the modern reader, *commutatio* is rendered as "parallax". On the other hand *obliquatio* is translated by the archaic "obliquation". I am not convinced that the flavour of the original is kept when *causa* is translated as "law" in such a context as Osiander's preface, as here: ". . . this subject [astronomy] is completely and simply ignorant of the laws which produce apparently irregular [planetary] motions". And I think that "correction" is an insufficiently specific rendering of *prosthasphaeresis.* But any careful reader of the text will not find much difficulty with technical terms.

The book is attractively produced at what must now be reckoned a very reasonable price, kept down by the expedient of printing the many figures in wide central margins. These are occasionally a trifle too small to be easily read, and are taken from the 1617 edition.

It must be noted that Dr Duncan's translation will soon be joined by another, produced by Edward Rosen for the Polish edition. The cost of the latter is likely to outstrip that of the David and Charles production, which is therefore more likely to reach the general reader, at whom the introduction and notes are aimed. Further comparison must be left until the Rosen translation is published. It is a moot point whether the market can take both versions, but English-speaking fans of Copernicus, working on the surgeon's principle that an extra opinion is always valuable, will no doubt insist that you cannot have too much of a good thing. ☐

J. D. North is Assistant Curator of the Museum of the History of Science, University of Oxford, UK.

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State of the Universe

Stuart Sharrock

The Key to the Universe: A Report on the New Physics. By N. Calder. Pp. 196. (British Broadcasting Corporation: London, 1977.) £5.95. *From Quarks to Quasars: An Outline of Modern Physics.* By S. Thomas. Pp. viii+294. (Athlone: London, 1977.) £3.95.

VERY few books deserve to be read. Some are memorable for the information they contain and others for the pleasure they give. It is a rare delight to find a book that fulfils both these criteria. On the whole Nigel Calder's latest offering, *The Key to the Universe*, is such a treat. At times the complexity of the subject is overwhelming and tedium sets in, but these occasions are few and forgiveable. Most of the book manages to convey the sense of excitement and achievement so sadly missing in the majority of texts on physics. It must be admitted, however, that reading this book can be hard work—the very subject matter ensures this—but those who persist will be well rewarded. The great advantage compared with the television programme presented by Nigel Calder on the same topic, is that the concepts can be taken in at a leisurely pace and the reader can refer back to items which were incompletely understood at first sight.

The Key to the Universe is an attempt to explain in layman's terms the recent developments in elementary particle physics and astrophysics which promise to produce a theory of the workings of the universe. Beginning with electricity and rapidly plunging into the weak force and gauge theories, quarks, colour and charm, the story moves on to gravity and exploding black holes, the Big Bang and broken symmetries. These concepts are described in plain language and the text provides an excellent introduction to current beliefs concerning the origin of the Universe, how it works and how it is evolving. Only a Philistine could claim that this topic is not of interest; here it is presented in a manner which is accessible and enjoyable.

Like all good adventure stories there is a hero—the physicist. Not a single individual but a series of people doing experiments and propounding theories. The potentially obscure concepts are illuminated by this emphasis on the personalities behind the ideas. But the story presented is one of almost unqualified success, and consequently sounds at times a little contrived; it would be more realistic and gripping if the villains also made an appearance.

My major reservation is the fact that there are insufficient explicit statements about the relative standing of different concepts. Ideas are given more or less equal weight irrespective of their current status. The experimental basis for charm is rather stronger than that for the decay of the proton, for example, and the unification of electromagnetism and the weak force is considerably more advanced than unification schemes involving gravity. The unwary reader may not recognise this.

The initiated—whom are not synonymous with the intelligent—will no doubt find details which irritate. The muon, for instance, may well just be a heavy electron but it has had its own identity for decades; I find the label "heavy electron" bewildering and sad. But this is a minor level of complaint about an attempt to explain the workings of the Universe in 200 pages.

As a chronicle of recent achievements presented in an exciting and responsible fashion this book is unrivalled; the concerned reader will gain access and understanding to an important part of our culture, but not without hard work. This book demands to be read.

From Quarks to Quasars is an entirely different style of book. The suggestive title is misleading as only 10% of the text is concerned with astrophysical phenomena, and the message of the book is not the connection between theories of the microscopic and the macroscopic. No attempt is made to be up-to-the-minute—charm is still a dream of the future and black holes do not explode.

What is presented here is a competent introduction to the atomic nature of matter at an elementary and descriptive level; the basic concepts are introduced and applied to provide a useful summary of the various states of matter: gases, plasmas, liquids and solids. Properties of the nucleus and elementary particles are discussed almost as in a catalogue of 'useful facts', and the chapters on quarks and quasars are pure appendages.

This is a physics book containing a large amount of useful, well-ordered and basic facts presented at an elementary level in a readable but unexciting style. It is ideal background reading for budding physicists about to start a university course; the uncommitted will find it useful but uninspiring. ☐

Stuart Sharrock is Physical Sciences Editor of Nature.

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Remarkable progress in astronomy

Bernard Lovell

Astronomy Transformed: The Emergence of Radio Astronomy in Britain. By D. O. Edge and M. J. Mulkay. Pp. xvi+482. (Wiley–Interscience: New York and London, 1976.) $31.75; £19.25.

HALF way through this book the authors state that they have completed their historical survey of the growth of radio astronomy. Their survey is primarily centred around the emergence of the new science in the UK covering the period from 1945 to about 1960—extended to 1963 to cover the discovery of quasars and, briefly, of pulsars in 1968. The authors state that they have chosen to base their description "around the development of research into discrete radio sources", and within this restricted timescale and framework they have pursued their aim with great determination. Supported by extracts from taped interviews with many of the participants, and by a most thorough series of references to the literature, a detailed account has been given of fifteen years of remarkable progress in astronomy.

In 1946, J. S. Hey published the evidence that a localised source of radio emission existed in the constellation of Cygnus. He and his colleagues had used the receiver section of an Army defence radar, and no-one could have foreseen the extent of the technical developments which were to follow during the next two decades leading to the discovery of thousands of discrete sources of radio emission. Edge and Mulkay provide a fair and largely unbiassed account of the succession of arguments and confrontations which by the end of their review period had led to a general consensus of opinion that the majority of these sources were extragalactic and that the measurements of their angular diameter and intensity distribution were of cosmological significance—even if the nature of the significance remained in dispute. After the pioneer work of Hey this story centres largely around the developments at Cambridge, Jodrell Bank and Sydney, and some of the participants will no doubt be surprised to be reminded how rapidly their opinions changed.

The meeting of the Royal Astronomical Society in October, 1951, was the occasion of a memorable exchange between Gold, Ryle and Hanbury Brown; and it is a pity that the full extract from this (like much other important material) has been relegated to appendices of notes and references which greatly increase the difficulty of a thorough study of the book. By 1951, although a few of the radio sources had been identified with extragalactic nebulae, Gold was almost alone in maintaining that the majority of the radio sources must be extragalactic. Even by 1957, when several thousand radio sources had been delineated, only eight had been identified optically; and three of these were associated with nebulosities in the Milky Way. By that stage the astronomical community was immersed in the contentious arguments about the differences between the Cambridge and Sydney radio source counts and whether they had relevance to the cosmological problem. Since Dr Edge was a graduate student in the Cambridge group for four of these remarkable years, he has been able to penetrate the details of a complex story, confused by the fact that, on the wavelengths then in use, the three strongest radio sources were near the galactic plane; but whereas two were supernova remnants in the Milky Way, the second strongest was a remote and peculiar extragalactic object.

The scope of the second half of the book is different. Here the authors investigate the relationships between the various groups of radio astronomers, the differing developments of the individual groups, the effect of radio astronomy on classical optical astronomy, and the extent to which the study of the emergence of radio astronomy contributes to the more general problems of the development of new areas of scientific inquiry. Parts of this will form compulsive reading for those who were in any way associated with these developments.

The bitter dispute over the radio source counts between the Cambridge and Sydney groups is summarised fairly. The remarkable secrecy maintained by Hewish and his colleagues over the pulsar discovery is described in relation to the problem of cooperation and competition, but the authors have balked the task of penetrating the Cambridge observational/theoretical conflict which has had such an influence on the present organisation of theoretical astronomy in the UK. In fact, their comment on this important matter is relegated to a footnote near the end of the book in which, referring to Hoyle, Gold and Bondi, they remark that the three "found themselves in Cambridge, but without any meaningful relationship to the small observational radio astronomy group, with whom they found dialogue difficult".

The most unsatisfactory feature of the book is the long chapter on *The Social Structures of the Groups* in which the authors "attempt to build up a picture of the evolution of the social structure and differentiation within the Cambridge and Jodrell Bank groups". They do this in a most peculiar manner—from "publication profiles, and co-authorship and citation statistics", and by quotation from anonymous members and ex-members of the two groups. The recollection of struggling research students of twenty to thirty years ago—however eminent they may be today—is scarcely a satisfactory means of making this investigation. In fact, I have difficulty in regarding this chapter seriously as a work of scientific or social investigation especially when after 57 pages the conclusion is reached that "the differences between the two groups can be adequately explained by postulating simply that Ryle and Lovell 'are two different people'. It is our view, however, that such a move is unnecessary". We *are* different people and it is those differences embraced within a framework of mutual understanding and collaboration about the problems of development in a society with limited resources which have enabled both of our groups to survive and develop.

This chapter suffers in comparison with the following chapter on *Some Sociological Implications*. This is by far the most important and valuable section of the book in which the innovation of radio astronomy is placed in the historical context and compared with the emergence of other new scientific disciplines. Particular comparisons are made with Ben-David's study of nineteenth-century innovations in medical science, of Mulkins' study of the orgin of molecular biology, and of the study by Dolby of the emergence of physical chemistry in the nineteenth century. This is a significant contribution to the scarce literature on a subject which should be of great concern to contemporary society, and is worthy of wider circulation than it is likely to obtain in this book. Clearly, Edge and Mulkay have condensed their information from an analysis of a great deal of published work and taped interviews; but further substantial sections could have been excised before publication, without detracting from a valuable historical narrative. ☐

Sir Bernard Lovell is Professor of Radio Astronomy and Director of the Experimental Station at the University of Manchester Nuffield Radio Astronomy Laboratories, Jodrell Bank, UK.

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Optical astronomy

Bernard Pagel

Sterne—Kosmos—Weltmodelle. (Erlebte Astronomie.) By O. Heckmann. Pp. 353. (Piper: München and Zürich, 1976.) DM36.

THIS book is a collection of essays by the most distinguished living German optical astronomer and theoretical cosmologist of what is now the older generation. Scientific and autobiographical material is combined to make an unusually lively and lucid account in non-technical (German) language of the developments in which he has played a leading role: cosmology, positional astronomy, star clusters, development of the Hamburg Observatory (of which he became Director in 1941 after a four-year interregnum due to reservations as to his acceptability by the Nazi authorities), and the foundation of the European Southern Observatory (ESO, of which he was the first Director General from 1962 until his retirement in 1969). There are also shorter articles, including an obituary of the great Walter Baade, a Presidential address on the 50th anniversary of the International Astronomical Union and some experiences that read like short stories.

In the section on cosmology (described with deceptive modesty as the author's 'hobby'), the most interesting chapter provides an account of the history of the subject, his own involvement therein, and its relationship to German politics in the Nazi era. In the face of the official and noisy campaign against "Jewish physics", Heckmann held seminars on special relativity and on cosmology in which he took pains to expose the futility of such distinctions; and in 1942 (by which time that campaign had lost some steam) he published an important monograph on *Theories of Cosmology* which gave clear expositions of Newtonian, general relativistic and kinematic cosmology, but nevertheless got past the censor (perhaps because its arrangement so clearly brought out the connections between them), earning international acclaim. After the war, Heckmann continued to make important contributions to the foundations of cosmology until deflected by the departure of his collaborator, E. Schücking, and his own involvement with ESO. He gives a very clear and thoughtful description, enlivened with personal experiences, of developments up to the present, together with further chapters on historical and philosophical aspects.

Heckmann's chief personal contributions to optical astronomy were in the study of open star clusters (largely in collaboration with H. Haffner), notably the precision colour-magnitude diagram of Praesepe. The astrophysical significance and the enormous work involved in reaching a degree of accuracy unprecedented in 1937, are very well described, although in this case the absence of any diagrams from the book is a weakness. At Hamburg, clusters continued to be a major preoccupation; another was positional astronomy—completion of the AGK2 and launching of the AGK3, which now provides proper motions and spectral types for 180,000 stars. Situated 20 km east of the city centre, Hamburg Observatory emerged from the War almost unscathed, although there was a narrow escape in the final battles around Lüneburg. After the War its staff was enlarged and its facilities enhanced with an 80-cm Schmidt telescope originally planned in 1937 in the hope of attracting Baade; it took immense political skill to get renewed funding in 1948. Significant studies of galactic structure were carried out with this instrument in the late 1950s, but its usefulness was already becoming restricted by city lights; and it has now been moved to the new Spanish–German observatory near Almeria.

The ESO project was suggested in 1953 by Baade as a means of redressing the balance both between the Northern and Southern Hemisphere and between US and European optical astronomy; to save time and money, direct copies of Californian telescopes were to be used. Governmental support was rapidly secured in Holland, Belgium, Sweden and West Germany, and later in France and Denmark; and the Ford Foundation contributed a million dollars. Progress was slow, however, until the 1960s when the site of La Silla in the Andes was finally adopted after extensive site-testing in South Africa and Chile. It now boasts a fine collection of instruments including a 3.6-m Ritchey–Chrétien, together with offices and laboratories in La Serena, Santiago and Geneva—a far cry from the quick project based on existing designs envisaged by Baade, but nevertheless a great achievement which owes much to the energy and foresight of its first Director-General.

Heckmann's book gives a rounded picture of a man who has served the scientific community well through his thoughtful intelligence, integrity and very considerable courage, together with outstanding scientific and international vision, applied with great administrative and diplomatic ability. It is well worth reading, also, as an authoritative and often engagingly frank account of some of the most important developments in cosmology and optical astronomy in recent years. ☐

Bernard Pagel is Deputy Chief Scientific Officer at the Royal Greenwich Observatory, and Visiting Professor at the Astronomy Centre, University of Sussex, UK.

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Nineteenth century kinetic theory

N. Feather

The Kind of Motion We Call Heat: A History of the Kinetic Theory of Gases in the 19th Century. Book 1: Physics and the Atomists. Pp. xxxix+1–302. Book 2: Statistical Physics and Irreversible Processes. Pp. xxxix+303–769. By S. G. Brush. (North-Holland: New York and Oxford, 1976.) Dfl. 210; $83.95 the set.

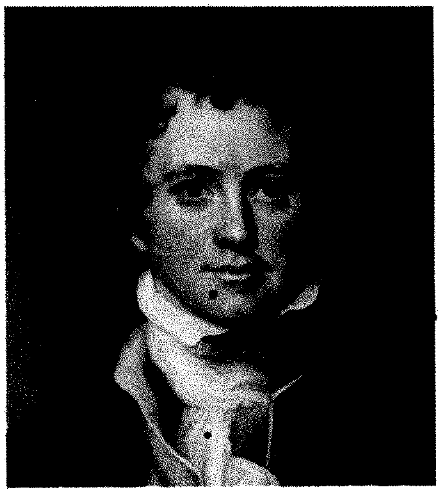
IT is not until he reaches p168 of this weighty monograph that the author admits that its title is taken, in literal translation, from that of Clausius' paper of 1857 which, as he says, "defined the scope and viewpoint of most 19th-century work in the kinetic theory of gases". Nothing else in the monograph is similarly derivative. Since he was invited to deliver a seminar at Harvard in 1954, Dr Brush has immersed himself in his chosen subject, and has written copiously about it. The present publication is a re-presentation and up-dating of the contents of 17 articles (the author says 15) which appeared under his name (one with C. W. F. Everitt as collaborator) in various journals between 1957 and 1974. These have been organised in two sequences. In Book 1 are accounts of the relevant contributions of seven individuals, from John Herapath (1790–1868) to Ernst Mach (1838–1916); in Book 2 discussions of seven 'problems' which exercised the minds of nineteenth century scientists and philosophers—problems ranging from "the wave theory of heat" to "Brownian movement". These two sequences are prefaced by a 100-page "introductory survey", specially written to give coherence to the whole work.

This is an interesting arrangement; however, as the author concedes, it "obviously entails considerable redundancy". "That may not be a disadvantage", he adds, optimistically, " . . . the reader can jump into the . . . chapters in almost any order . . . without losing too much of the overall picture". Unhappily, such licence is not vouchsafed a reviewer—although when he meets an outline of the derivation of Boltzmann's H theorem for the third time he is tempted to skip.

The chapters are divided into sections, and footnotes are collected together at the end of each section. There are some 108 sections so annotated, and with the number of sectional footnotes ranging from 1 to 59, it will be clear that the publishers' claim that "the author's historical researches have gone far beyond any other books on the subject" has at least statistical justification. Moreover, the last 49 pages of the monograph contain a separate bibliography, with brief indication of content, of 539 publications, listed by authors arranged alphabetically. These publications, Dr Bush considers, constitute the essence of the matter, so far as the nineteenth century is concerned. He has cited "thousands" of others in the footnotes—but he admits that they are "minor works".

What, then, has this monumental effort achieved? A balanced account of the history of the development of the subject within the century surveyed—that cannot be gainsaid—and a redistribution of emphasis and credit. Credit to Herapath and Waterston for being in advance of their time (largely because the notion of heat as a wave process, which became popular in the early years of the ninteenth century, went far to diminishing the impact of the experimental researches of Rumford and Davy). Similar credit to Leslie as the first to distinguish the three modes of heat transfer. Credit to Loschmidt and E. P. Culverwell for pertinent criticisms which led Boltzmann to a deeper study of his statistical theory. And, in respect of the last years of the century, a reappraisal of the discontents which beset kinetic theorists generally—the problem of gaseous specific heats seen as a minor problem, in comparison with more fundamental doubts of a quasi-philosophical character concerning randomness and irreversibility.

No-one will read Dr Brush's account without having his horizons widened and his detailed knowledge of the history of the subject immeasurably increased. It must be said, however, that he is likely to be much irritated in the process. Leaving aside the 'redundancies' in text and footnotes (it would seem almost as if the field is wide enough to sustain an ergodic theorem in respect of the continuing theme of this book), he will be irritated by the numerous textual errors and misprints.



Experimentalist Humphrey Davy

Photo: Mary Evans Picture Library

As a matter of interest, I checked the examples noticed during a single reading of chapters 11 and 12 against the relevant articles in *American Journal of Physics* where the material was originally published. I found three cases in which a typographical or textual error was repeated from the original version, and fourteen examples of errors newly appearing—five in new matter, and nine in places where there had been no error before. One example of the latter class is particularly puzzling. On p413, the factor $(1+(b/v))$ in equation (13)—which was correct—has been replaced by $(1-(b/v))^{-1}$, although the whole point of the argument in the following paragraph turns on the retention of the correct form in the equation.

There is an interestingly gratuitous error in the introductory survey (p43). Traversing a field of physics less familiar to him than gas theory, the author is presenting the old story of Fresnel and Poisson and diffraction by a circular disc. Poisson, he says, concluded that, if Fresnel were correct, "there should be . . . a tiny bright spot at a certain distance behind the disk". When the test was made, "the bright spot appeared just where the mathematics predicted".

In a work of the magnitude before us, occasional errors are inevitable, but, sadly, there are more than they should be; and some will almost certainly mislead. American usage, too, is sometimes subtlely different from our own. One example will suffice. Cayley is described as a 'colleague' of Tait (p358). Cayley, however, had left Cambridge before Tait went up as an undergraduate; by the time he returned, Tait was already in Edinburgh. They were never workers in the same institution. This usage, however, seems not to be accidental. In his preface, Dr Brush has a list of 22 "colleagues", the world over, whom he thanks for "valuable suggestions and information". At least he does not call his helpers "co-efficients", as Kelvin did (preface, *Baltimore Lectures*). Incidentally, Kelvin's nephew, J. T. Bottomley, worked, not "in England" (p520), but in his uncle's laboratory in Glasgow—and Oliver Lodge published (in 1894), neither from London nor from Birmingham (p745), but as professor in Liverpool. ☐

N. Feather is Emeritus Professor of Natural Philosophy at the University of Edinburgh, UK.

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Perspectivist tradition leads men of vision

J. D. Mollon

Theories of Vision from Al-Kindi to Kepler. By D. C. Lindberg. Pp. xii+324. (University of Chicago: Chicago and London, 1976.) £13.60.

In laboratories and coffee-rooms it is countless times debated whether a knowledge of the literature allows the scientist to stand on the shoulders of his predecessors or merely bestows on him a *damnosa hereditas*, fatally constraining his thought. The literature man scorns the rediscoveries and the omitted controls of his unread colleague: the latter argues that it is often faster to do the experiment than to find the answer in the literature and points to the low publication rate of the literature man.

Two of the greatest contributors to our understanding of visual perception, Thomas Young and Hermann Helmholtz, were literature men of enormous erudition whose systematisations too easily conceal from us the achievements of the eighteenth century. In his book, D. C. Lindberg argues that a third outstanding innovator in the theory of vision, Kepler, was not a revolutionary working in a vacuum but rather represents the culmination of the perspectivist tradition that extends back to the Islamic writers of the ninth and tenth centuries. He shows that Al-Kindi first made explicit the principle that luminous rays issue in every direction from every point on the surface of a luminous body and that Alhazen set the problem, that of establishing a one-to-one correspondence between points in the visual field and points on some plane in the eye. The pre-Keplerian solution was to assign an especial potency to those rays that were perpendicular to the surface of the cornea, that is, to those making up the visual cone, with its base on the object of vision and its apex at the centre of curvature of the cornea. (Lindberg does not notice that this theory is lent some truth by the Stiles–Crawford effect, the directional selectivity of the photoreceptors that favours rays normal to the retina). Kepler, equipped with the improved anatomy of Felix Platter and with his own understanding of the focusing properties of transparent spheres, was able to offer the correct solution, that the crystalline humour is not itself the seat of vision but in combination with the cornea serves to form an inverted image on the retina. But, Lindberg argues, the perspectivist tradition gave Kepler his problem, his facts, his methodological rules and his criteria of success. In this conclusion Lindberg explicitly opposes the standard view that Kepler transformed visual theory by mechanising it: "Kepler presented a new solution (but not a new kind of solution) to a mediaeval problem, defined some six hundred years earlier by Alhazen".

Lindberg himself is clearly a literature man by temperament. His impressive grasp of the primary Latin texts is complemented by a very wide knowledge of the academic literature: his book has over 1,000 footnotes to secondary sources. So it is understandable that he is unimpressed by Leonardo's contribution to visual theory. Leonardo was avowedly not a literature man and himself wrote: "If indeed I have no power to quote from authors as they have, it is a far bigger and more worthy thing to read by the light of experience, which is the instructress of their masters. They strut about puffed up and pompous, decked out and adorned not with their own labours but by those of others, and they will not allow me my own". Lindberg believes that Leonardo had read Pecham and Witelo but that his knowledge of the optical tradition was imperfect. Usually Leonardo did not work with Al-Kindi's principle of punctiform analysis but rather thought of the species, or image, of an object as radiating holistically. His understanding of the formation of images by reflection and refraction was limited. It is particularly curious that this skilful anatomist was so weak in ocular anatomy: he confounded the vitreous and the crystalline and supposed that the eye consisted of two concentric spheres. By reading any of the mediaeval perspectivists he would have been furnished with a more sophisticated account. Lindberg is mistaken, however, in admonishing Leonardo for claiming that the apparent size of objects increases with the size of the pupil: there is a clear basis for this observation in the phenomenon of irradiation. Leonardo's *explanation* is another matter, but it is noteworthy that his claim about the effect of pupillary size is contained in the same manuscript (F of the Library of the Institut de France) as his observation that a uniform iron rod heated only in part of its length will appear considerably larger in its heated part. The latter phenomenon is primarily a consequence of irradiation.

The theory of optics and vision was central to mediaeval philosophy and provided a link between physics, medicine and theology. Optics was, for example, fundamental in Bacon's planned synthesis of all human knowledge. Lindberg's book should therefore be of interest to mediaeval historians in general. He draws attention, for example, to the neglect of the history of the translations from Greek and Arabic to Latin in the twelth century: almost nothing is known of the purposes and motivation of the translators, of the selection of works or of the mechanics of translation; and towards the further understanding of this crucial movement, Lindberg provides a detailed Appendix on the translation of optical works.

The book is illustrated with many diagrams of the eye drawn from ancient and mediaeval authors. Man's idealised representations of his own visual organ serve nicely to illustrate how his perception is influenced by his conceptual framework. Perhaps we can never accurately perceive a biological mechanism until we have constructed the artefact that will serve as a model: we must have lenses before we can perceive the eye as an image-forming device and computers before we can perceive the brain as an information-processing machine. What is regrettable is that Lindberg nowhere includes an accurate modern diagram of the eye, an inclusion that would have been invaluable to the general reader trying to follow mediaeval discussions of whether there are four tunics or seven, three humours or two.

To reduce his material to manageable proportions, Lindberg omits discussion of theories of the physiology and psychology of vision. To some extent this exclusion is artificial, for it is clear that many of Kepler's predecessors (including Leonardo) were held back from the correct solution by explicit reluctance to entertain an inversion of the image; and Kepler himself acknowledged that he "dutifully tortured" himself "in order to show that the cones intersecting when they pass through the aperture of the uvea intersect again behind the crystalline, in the middle of the vitreous humour, so that another inversion is produced." But he protested that geometry allowed no escape from an inverted image and thereby bequeathed to his own successors a new and peculiarly vexatious problem. ☐

J. D. Mollon is Lecturer in Experimental Philosophy at the University of Edinburgh, UK.

Mental imagery

John Krebs

The Question of Animal Awareness. By D. R. Griffin. Pp. viii+135. (Rockefeller University: New York, 1976.) $8.95.

GRIFFIN'S thesis is that animals experience mental images and therefore a primitive form of awareness. One could argue about the meaning of these words and their relationship to the more strictly human experiences of subjective emotions and self-awareness, both of which are specifically excluded from Griffin's definitions. But since it is easy to see roughly what Griffin is getting at, I think such a philosophical argument is superfluous. Much of the book is filled with the 'evidence' for animal awareness, and although the style is clear and concise, I feel that Griffin makes an unnecessarily elaborate case: most behavioural biologists are quite ready to accept the idea that animals have mental images of events or objects in their environment.

The main points of Griffin's thesis are as follows. First, he establishes, with a few examples—such as bats catching their prey by sonar and birds orientating by star compasses—that animals are rather more complex than might have been supposed 50 years ago. Especially remarkable are the linguistic abilities of chimpanzees and honey bees. Chimps, such as Washoe, can construct sentences in American Sign Language, showing that they understand a few rules of human language, and bees use an arbitrary communication system for encoding information about remote feeding places.

Griffin sees the complexity of animal language as important, because so much of man's mental experience is dependent on language. (As an aside, the chimp and bee examples are always quoted as evidence for the complexity of animal language: chimps one can understand, but bees seem to be an anomaly. Either they are an evolutionary freak, or we are waiting for more scientists of von Frisch's genius.) In fact, the complexity of animal language does not seem to be a crucial part of Griffin's thesis, since some of the clearer examples of mental imagery in animals—for example, 'searching images'—do not involve complex language.

Another line of evidence used by Griffin is neurophysiological. He revives the argument that the nervous system of man is only quantitatively different from that of animals, so products of the nervous system such as awareness should also differ only in quantity. This assumes, of course, that quantitative differences are not translated by thresholds into qualitative effects.

Finally, Griffin argues on evolutionary grounds that mental awareness has survival value in allowing an animal to anticipate and cope with complex problems, so it should be favoured in all animals by natural selection. Interestingly, Griffin does not use dreaming in animals as evidence for mental imagery, although all dog-watchers will agree that dogs dream about chasing the cat next door.

Whether or not they accept the individual steps in his argument (most of which are not new), most behavioural biologists will concur with Griffin's overall thesis. Equally, I feel that most will be disappointed with Griffin's answer to the "so what?" question. The test of the value of any scientific hypothesis is whether or not it generates new experiments, and it seems to me that the awareness hypothesis fails this test.

Griffin argues, by analogy with man, that the only way to find out about an animal's mental experiences, it to ask it directly, or indirectly, what is it thinking about. With Washoe this might be done through American Sign Language, with bees and birds through life-like dummies or models. If one leaves out the special case of Washoe and his clan, what it boils down to is a plea for more work on animal communication. This is an uncontroversial proposition, but if I try to think up experiments or observations to elucidate the meaning of, for example, the incredibly complex song of a skylark, is the hypothesis that larks have mental experiences going to help me? ☐

John Krebs is Lecturer in Zoology at the Edward Grey Institute of Field Ornithology, University of Oxford, UK.

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Brain anatomy

J. Z. Young

The Vertebrate Brain. By R. Pearson and L. Pearson. Pp. xi+744. (Academic: London and New York, 1976.) £23.

THE great expansion of interest in neuroscience in recent years has not been accompanied by an equivalent interest in comparative neurology. Microscopists and experimentors have indeed used neurones from very diverse animals, but few have tried to investigate their specific adaptive features in relation to the environment. Still fewer have tried to follow the course of evolution of the brain throughout the vertebrate phylum. The only comprehensive work on the subject is that of Kappers, Huber and Crosby written in 1936. The leading living expert in the field in Hans Kuhlenbeck, whose major work is still appearing. One looks therefore with excitement at a new book on *The Vertebrate Brain*. The authors' aim is to provide "a survey of the anatomical, biochemical and physiological aspects of the vertebrate brain". They believe that the development of the neurosciences "will probably prove as profound in their effects on man and his philosophies as did the concepts of nuclear physics during the 1930s or the 'molecular biologists' of the 1950s".

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of the literature of the classical comparative anatomy of the cell masses and tracts in different vertebrate brains. It is a formidable problem, indeed, to know how to present this knowledge. The authors have decided to take each part separately. So we go through the details of the medulla from Agnatha to Mammals and then start on the cerebellum, and so on. It is not the authors' fault that such a division makes for difficulties in following particular functional systems. In some parts it works well—for instance, the mid-brain roof—but it is disastrous for the reticular system, which is scattered all through the book, and it does not work well for the forebrain.

No-one has discovered the principles with which knowledge about the structure and functioning of various nervous systems can be compared. The authors' brave attempt provides some of the facts and the stimulus to attack the problem. ☐

J. Z. Young is Emeritus Professor of Anatomy at University College, London, UK.

Perceptual exploration

Stuart Sutherland

Cognition and Reality. By U. Neisser. Pp. xiii+230. (Freeman: San Francisco and Reading, 1976.) £3.70.

In 1967, Neisser published a book called *Cognitive Psychology*: it was a vigorous and clear account of that subject which marked its coming of age. The book was optimistic and gave the impression that we would eventually come to understand the workings of the mind in terms of the information processing operations it carries out. In *Cognition and Reality,* Neisser seems to have become somewhat disillusioned with the progress of the cause he espoused ten years ago. Workers in the field, he claims, are trapped in specific experimental paradigms; they fail to tap the true potential of the human mind since they use unpractised subjects and artificial tasks; their work has little bearing on real life situations and does not tell us what mind or consciousness really are.

Neisser's own solution to the problem of cognitive psychology takes the form of a shot-gun marriage between J. J. Gibson and F. C. Bartlett. To understand the mind, we must first understand the world in which people live: in particular we must understand the relationship between the information available to our senses and the external world specified by that information. The information we "pick up" depends on our internal schemata: they guide perceptual exploration and are in turn modified by it. Perception is not an instantaneous happening but an ongoing interaction between our schemata and the world. "Images are not pictures in the head, but plans for obtaining information from potential environments".

The book makes some cogent points even if they are often incidental to the main theme. For example, Neisser points out that except in a very constrained environment where people have few choices, we shall never be able to exert behavioural control over other people: to do so, we would need to have a complete understanding not only of them, but also of the world in which they live. We cannot predict, let alone control, the moves a master chess player will make unless we have at least as much knowledge of chess as he does. Elsewhere, Neisser exposes some of the crudities of earlier theories of cognition. He has himself shown, amongst others, that with sufficient practice it is sometimes possible to carry out two complex tasks at once: the brain is not in any obvious sense a limited capacity channel.

Cognition and Reality is not an easy book to understand or to evaluate. First, it is unclear how far Neisser is putting forward a new thesis rather than merely using a different terminology. Nor is it obvious whose views he is attacking. There is a good deal of verbal fencing: for example, he registers his objection to the idea that the mind *processes* information by writing: "The information itself is not changed, since it was in the light already. The schema picks it up, is altered by it, uses it". Second, his exposition of the operation of schemata remains at a very general level and he is curiously reluctant to give examples of how his ideas can explain any phenomena in detail. When he does attempt explanation, as, for example, in his discussion of the perception of emotional expression in others, the explanations are at such a general level that it is hard to see whether any new light has been thrown on the problem.

At times one may even suspect that the constant flourishing of the magic word "schemata" serves more to distract attention from the phenomena to be explained than to explain them. Although the status of Neisser's central thesis remains unclear, the book deserves to be widely read if only for the interesting and acute observations that are scattered through its pages. ☐

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex, UK.

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Technology and food production

C. R. W. Spedding

Food Production and Consumption: The Efficiency of Human Food Chains and Nutrient Cycles. Edited by A. N. Duckham, J. G. W. Jones and E. H. Roberts. Pp. xx+541. (North-Holland: Amsterdam and Oxford, 1976.) Dfl.120; $48.95.

THIS is a bold and ambitious book that deserves to be treated as a serious attempt to encompass in one volume the entire food production system of the world, from photosynthesis to marketing and consumption. Rigorous criticism is therefore a better use of limited reviewing space than mere description of contents. The latter can be summarised as being organised into 28 chapters (by 31 authors) in five parts (introduction; ecological aspects; biological components; social and economic aspects; a strategic synthesis). The book is well produced and edited, and relatively free from minor errors.

The quality of the individual contributions is inevitably uneven but as a compilation of basic facts the book is extremely valuable, although more could usefully have been included on, for example, the inefficiencies of animal populations and nitrogen fixation in plants. The enterprise, however, was intended as much more than a compilation; what has to be assessed, therefore, is the success of the synthesis achieved.

Energy is used as "the major currency" and, although the arguments are strong, it is perhaps rash to base a total synthesis too heavily on this alone. It is argued that the value of land depends largely on its ability to derive an income from solar energy. The world is, however, full of exceptions to this: the very ability referred to is dependent on other inputs with costs that have to be justified by probable profit and this is often not related at all to current energetic efficiency.

Furthermore, there are dangers in regarding food as merely a biological necessity. Space, water and oxygen are also biological necessities, but who would accept an argument based on providing the minimum biological requirements for these necessities? If man wants more than the minimum out of life, 'better' food would seem at least as reasonable an optional extra as cars, bingo or television.

Indeed, the book recognises that there is little hope of diverting cereals from livestock even though this could result in more food being available. The question of "available to whom?" remains a worrying one. The confidence generated by the conclusion that there are "no insuperable biological problems in feeding the world up to 2000 AD" evaporates somewhat when it is realised that both hunger and overconsumption now exist independently of technical knowledge, even when applied.

The conclusion that food systems are extremely inefficient is well documented but there is, in my view, too little emphasis on the problems of tackling such inefficiency. Identifying a problem has to be followed by selection, from all the technical possibilities, of an appropriate solution.

Although a systems approach is advocated, the book is mainly based on an examination of food chains—linear linkages without feedbacks. The editors recognise this but do not greatly emphasise that, when governments are recommended to focus on particular inefficiencies, they should do so with due regard to all the consequences. The difficulties of doing this ought to be amongst the reasons for the modelling activity recommended.

It will certainly be necessary, as is strongly argued, to aim at high yields *without* high inputs of support energy, but this may not be sufficient. No-one would quarrel with the conclusion that "the greatest potential improvement thus lies in the area of conventional agricultural technology", but this is always likely to be so in the short term (if we mean by improvement greater food production). But if much more food was grown and harvested, the dilemma would remain, that the hungry people are also desperately poor.

Despite these criticisms, this volume is a constructive step in the right direction and will be of great value to all those interested in the application of technology to efficient food production.

C. R. W. Spedding is Professor of Agricultural Systems and Head of the Department of Agriculture and Horticulture at the University of Reading, UK.

Foresight and responsibility in land use

John Andrews

The Thames Transformed: London's River and Its Waterfowl. By J. Harrison and P. Grant. Photographs by P. Harrison. Pp. 240 (André Deutsch: London, 1976.) £5.95.

INDUSTRIALISED British estuaries have recently begun to receive the attention necessary to retain or restore their natural productivity. The Thames exemplifies the work, its results and the complex problems to be solved if the international importance of wildlife in our major estuaries is to be maintained.

One-third of *The Thames Transformed* deals with the inner estuary, describing well the decline of water quality to the point where the river was a deoxygenated broth in which neither bird, fish nor weed could persist, and outlining the remarkable work of the London County Council/Greater London Council and the Port of London Authority in restoring reasonable conditions in which wildlife could return. Ironically, continued improvement has now reduced the massive *Tubifex* population on which the wildfowl feed, so that their numbers are declining, whereas waders have lost feeding areas due to reclamation.

The authors are experienced amateur ornithologists, Jeffery Harrison being known particularly for work on wildfowl and his concern for the north Kent marshes. Nearly half the book is devoted to this area—the Essex shore is dismissed in a cursory eighteen pages—and deals almost exclusively with wildfowl, including detailed historical accounts of occurrences. There is no discussion of the ninety-thousand waders or other bird populations, nor of the botanical, entomological and other important scientific features of the outer estuary. Concentration on the wildfowl produces recommendations about the agricultural management of the marshes which are not in the best interests of the total biological resource, whereas the conflict between wildfowl—particularly brent—and farmers, is glossed over. The identification of current industrial and recreational threats is valuable but too brief to demonstrate fully the complexity of the problems, both from the point of view of the 'developer' faced with real social and economic issues, and of the 'conservationist' striving to maintain scientific interest.

The authors suggest that the north Kent marshes should become a National Park rather than a National Nature Reserve, perhaps because the Nature Conservancy Council (NCC) has never had the finance required. Indeed, the money for research and land acquisition which resulted from the Maplin Airport threat, were gained for NCC by the voluntary bodies which fought the Bill at Committee stage. It seems odd that there is no mention of this and some other important milestones, including the establishment of the NCC/Ministry of Defence Advisory Committee on Foulness and of the Thames Estuary Wildlife Conservation Group. The authors do give credit, however, to the conservation efforts of the PLA, the Central Electricity Generating Board, and others. By encouraging them, their book can only help conservation which depends so much on the foresight and responsibility of all involved in land-use decisions. ▣

John Andrews is Head of the Conservation Planning Department of the Royal Society for the Protection of Birds, dealing with land-use issues affecting birds throughout the UK.

Baffling disease

C. H. Andrewes

Influenza: The Viruses and the Disease. By Sir Charles H. Stuart-Harris and G. C. Schild. Pp. x+242. (Arnold: London, 1976.) £12.

THE remarkable properties of the causative virus have made influenza a most fascinating yet baffling disease. Despite a vast amount of work there is still much to learn, particularly about how the infection can be controlled. Stuart-Harris and Schild's book is packed with information. For much of his working life, Sir Charles Stuart-Harris has been involved with the disease; he has in particular cultivated the area in which clinical and laboratory medicine overlap. Dr Schild was until recently in charge of the WHO World Influenza Centre at the National Institute for Medical Research, London, UK. Each of 12 chapters is followed by a list of references varying in number from 40 to over 100. Even these, as the preface points out, represent only a selection from the vast world literature.

We have first an outline of "the modern history of the study of the causes of human influenza". The authors pay only brief attention to work preceding the discovery, in 1933, of the susceptibility of ferrets to infection. Indeed there is only passing mention of Pfeiffer's so-called influenza bacillus, *Haemophilus influenzae*. Nor is much space devoted to outbreaks in earlier years, for the identity of the infecting agent of those times can only be inferred. Even the catastrophic 1918–19 pandemic is not considered at length.

Having reviewed the most important laboratory findings, including transmission to animals, cultivation and interference, chapter 2 discusses in great detail the molecular biology and replication of the virus. There are many excellent and well reproduced electron micrographs. These depict the whole virus and its separated components, and are supplemented by diagrams clearly explaining the structure and mode of replication of the virus.

Description of the viral antigens leads on to discussion of virus variation, the minor changes known as 'antigenic drift' and the periodical major 'antigenic shift' that herald pandemics. We learn how two viruses growing together in culture or in living animals may exhibit recombination of characters, resulting in viruses with hybrid properties.

Chapter 4 deals with the influenza viruses of swine, horses, birds and other species; for it is now recognised that man is only one host of a versatile family of viruses. There is increasing interest in the possibility that new pandemic strains may arise from recombination between human and animal viruses. The authors are, however, very cautious and write: "At the present time is seems somewhat premature to assess the significance of these findings . . .".

Chapter 5 describes the disease in man and its complications. We then have two chapters on epidemiology, with numerous diagrams illustrating the variations in mortality and morbidity from year to year. The authors admit to being puzzled as to where the virus goes between outbreaks and why older viruses disappear when new antigenic variants appear. There are full references to the work that led to the doctrine of "original antigenic sin". This concerns the remarkable fact that a person's first experience of a virus determines the pattern of his subsequent antibody responses: these are largely directed against the virus he first encountered rather than against more recent ones. When tests are made in the light of this, against various animal viruses, all sorts of intriguing questions arise about the nature of the influenza viruses involved in outbreaks of earlier years.

Studies of immunity, next described, deal largely with the relation between immunity and the level of antibodies in serum and in nasal secretions. Clearly it is vital to understand these matters if vaccination is to be attempted with any success. The results of several trials with inactivated vaccines of various kinds are reviewed in detail, and there is discussion about the best strategy for vaccine administration.

There are obvious theoretical advantages in the use of a live attenuated vaccine: it can be given as a spray and thus be much more acceptable; and it would be vastly cheaper. Methods used for virus attenuation are described together with the results of trials; those on a large scale have been carried out mainly in the USSR and Jugoslavia. It seems that, although acceptably attenuated vaccines are available, the results of trials have been variable. The authors suggest that live vaccines are more likely to be effective "when a new pandemic virus is triggering off a new cycle of epidemics". Otherwise, killed vaccines may be better.

"Some progress" is reported in the field of chemoprophylaxis and chemotherapy. Amantidine, in particular, is discussed, as is the theoretical basis of the ways in which drugs may contrive to upset replication or release of virus.

The final chapter provides details of the various techniques in use for the isolation and assay of virus, and of the various serological methods in use. Particularly useful will be the accounts of the recently described "single-radial-immunodiffusion" and "single-radial-haemolysis" tests.

A postscript discusses the recent excitement over the small outbreak due to a swine-like virus in New Jersey. As is wisely pointed out, "spread of swine influenza viruses from pig to man and their restricted spread in the population may have occurred from time to time in the past without detection".

The book will be of great value to workers in this field and to those concerned with preventive measures. It invites comparison with another, much smaller, popular book on the subject, *Influenza, the last great plague* by Professor W. I. B. Beveridge (Heinemann Educational: London, £2.95). Stuart-Harris and Schild's book is full of well documented facts, but the authors are very chary of even modest speculation. Not so, Professor Beveridge; he freely discusses pandemics from 1700 onwards, considers the birth of pandemics, and seems to have little doubt about the role of animal viruses in their genesis. Whereas one book describes the "trees" in most valuable detail, the other makes a bold attempt to discern the nature of the "wood".

The only serious criticism of Stuart-Harris and Schild's book is that the print is annoyingly smaller than usual; doubtless the price would otherwise be even higher. One hopes that the rising cost of books will not cause publishers to lower their standards of presentation.

Sir Christopher Andrewes was Deputy Director of the National Institute for Medical Research, London, UK, from 1952–61, during which time he was also in charge of the WHO World Influenza Centre.

Climatic pipe dreams

R. P. Pearce

The Cooling. By G. Ponte. Pp. 306. (Prentice-Hall: Englewood Cliffs, New Jersey, 1976.) $8.95.

THE author of this book is described on the fly-leaf as having been an arms control researcher and who has since worked as a television and radio commentator. The book is about world climatology, its scientific as well as its wider human aspects. I, the reviewer, am a meterorologist who has spent a large part of his life in a University environment, attempting to solve some of the enormously difficult scientific problems which the atmosphere poses, of which those relating to longer-term climatic changes on the global scale are perhaps the most challenging of all.

I can only describe the experience of reading this book as having been something resembling a meteorological nightmare. Such a hostile instinctive reaction to a book makes it difficult for any reviewer to present what he feels he will regard in retrospect as a fair and reasoned appraisal, and I will therefore make every effort to give the author as much credit as it seems possible to give him, bearing in mind the highly controversial nature of the topic on which he has chosen to write.

The first part is an account of existing knowledge of climatic trends, particularly those of the past 20,000 yr or so, and of the physical factors determining climate. The presentation emphasises the role of solar radiation and discusses processes likely to result in variations of solar energy output. The second part examines the influence climatic factors have had on human activity from prehistoric to more recent times. The increasing susceptibility of the rapidly expanding and technologically developing human population to climatic variability is strongly emphasised; an appreciation of this by governments is illustrated by their involvement in discussions of schemes to control weather in various ways—for example, by hurricane modification, rain enhancement by cloud seeding, and the construction of barriers to modify ocean currents. Illustrations are also drawn, mainly from the US experience in Vietnam, of the use of weather modification as a weapon of war. The severe consequences of adverse weather such as droughts and hailstorms on food production over large parts of the globe are discussed in some detail, and the activities of weather modification groups to alleviate the worst effects of these phenomena are given some prominence. The legal aspects of these activities are also discussed.

The third and final part of the book looks to the future. Taking as its basic proposition that there is a one-in-ten chance that the Earth's climate will cool and become more hostile to and seriously affect human activity over a large part of the globe within the next hundred years, it examines in some detail three possible options (apart from doing nothing) open to us for meeting this situation. The first is to adapt ourselves to the anticipated new conditions (for example, by introducing mandatory birth control to stabilise the world population, accompanied by food, water and energy rationing): the second is to try to modify climate locally—for example, by building a dam across the Straits of Gibraltar; and the third is to try to alter the global climate by, for example, blackening the polar ice caps or damming the Bering Straits. The author, after a brief reference to present international research efforts under the Global Atmospheric Research Program and stressing the urgent need for more research, concludes that the wisest course is a flexible mix of options.

The work is written in a highly sensationalist style which never lets up. The author's insatiable urge to impose his ideas on the reader and saturate him in his verbal imagery frequently leads him to use phrases which are logically absurd and amount to little more than scientific mumbo-jumbo. An example of this occurs in his description of a stationary high pressure area over Greenland as "causing a slight vacuum effect in wind patterns that increased cold winds sweeping down into Europe". Another is his description of tornadoes as "masses of cold air bumping into each other in thunderstorms". Some of his diagrams, too, are inaccurate. One of these shows the intertropical convergence zone as penetrating north of the Himalayas and up into Mongolia during the period 1900–1940!

But there is another aspect of this book which I find highly irritating and irresponsible. This is its almost total disregard and arrogant dismissal of the substantial progress made by the worldwide scientific meteorological community in recent years in getting to grips with the fundamental problems concerning the physical basis of climate. The Global Atmospheric Research Program gets a mention here and there, but this hardly compares with the attention given, for instance, to the work of those who predict that an ice age could be upon us in seven years, or who talk of "punching holes" through the ozone layer with laser beams as a possible means of waging war on an enemy. The US National Academy of Sciences has recently produced a highly professional and valuable report (*Understanding Climatic Change: A Program for Action*) but this is referred to only briefly in an Appendix; and the Academy itself is cursorily dismissed as a "staid group".

All this is a great pity and, I fear, could even be dangerous. Lay individuals reading this book (including politicians, one of whom has contributed a foreword) could easily be misled into thinking that meteorologists know enough about global atmospheric behaviour to make a reasonable guess at what will happen to our climate over the next hundred years; and that we are on the verge of being able to change climate to provide a more favourable environment for mankind. The impression is given that only those meteorologists prepared to indulge in climatic prediction and weather modification have any real concern for applying their science to the benefit of humanity and that the others are just sitting back and taking little effective action.

It is a pity that the author has not directed his obvious talents as a publicist primarily towards drawing the attention of those who read his book to the great economic importance of weather and climatic research and to the need for strong financial support for the activities of those who are, and have been for many years, attempting to fill the large gaps in our scientific knowledge of the atmosphere and oceans, and the factors determining their behaviour, particularly on the longer time scales. Contrary to the impression conveyed by the author, most meteorologists see these as the primary goals of the science at this stage in its development. Until we have made more progress in this direction, reliable climatic prediction and control, the main themes of this book, are but pipe dreams. □

R. P. Pearce is Professor of Meteorology and Head of the Department of Geophysics at the University of Reading, UK.

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British weather

H. H. Lamb

The Climate of the British Isles. Edited by T. J. Chandler and S. Gregory. Pp. xviii+390 (Longman; London and New York, 1976.) £5.95.

THIS useful book is likely to win wide acceptance as the fundamental reference work that its title implies. It presents a mine of information within a handy size and will serve many practical needs. Its editors, who are the heads of the Georgraphy Departments of Manchester and Sheffield Universities, planned the book as a successor to E. G. Bilham's classic work which appeared under the same title in 1938: this new version, written with more awareness of climatic change, and incorporating a chapter on that topic, is to provide up-to-date observational data, including much (for example, on pollution) that was not available 40 years ago, and an introduction to modern ideas and analysis techniques (notably the greatly increased use of synoptic climatology). Although 12 of the 15 chapters are by scientists holding university appointments and only three by present or recently retired members of the UK Meteorological Office staff, the Office, together with the Irish Meteorological Service and their official publications, have provided most of the data.

The book is on about the same scale as Bilham's, having 390 pages to his 347 and 110 maps and 80 tables to his 101 and 63. Like all works made up of contributions from different authors, however, the style and standard of the chapters in the new work is somewhat uneven (although as regards most, not badly so). One is not told about the qualifications of the authors, but the research fields preferred by all but two of them can be recognised in their works cited in the 560-item bibliography (a very good list of modern references, although omitting some of C. E. P. Brooks's works, such as his *Climate in Everyday Life* and *British Floods and Droughts*, which are still useful).

It is sad to have to criticise anything in such a useful straightforward job as this compendious book. Yet the text is presented in a thin spider's web of print which is also too large for the spacing of the lines and fades to an indecipherable greying of the page in poor light in which Bilham's older text is still clearly readable. Another unhappy choice is the bold poster-print of page-numbers in the index which has no point in combination with the spidery print of the subjects to be identified. The style in itself is not unattractive but is more appropriate to a mail-order catalogue than to a book that is to be read and re-read carefully over many years. By contrast, the printing of the many maps and tables is mostly good; but it is too small, so that a magnifying glass is needed to check many details. In some cases this, combined with awkward spacing of the figuring (as in Fig. 6.6 on p141), presents a real danger of misreading 1.0 as 10 and 2.5 as 25, and so on. It is odd, too, that so few publishers seem to realise the virtue of giving the key date—the year(s) and/or month or season—of each map of a series boldly on the map rather than in a list in small print in the group caption. There are also a few complaints about language. The Gaelic word Eire, which means the whole island of Ireland, is wrongly used in the title of the Irish Meterorological Service and elsewhere in the book to relate to just the 26 counties of the Republic. Another author prefers to use the five-syllable word "altitudinal" when he means "height" differences. In these changing times the fashion for changing names presents problems to writers who have to refer to the geography. This book boldly uses names of new counties in England and Wales, possibly before it is certain that they will stick.

In writing of the book's many virtues it is hard to know what to stress. This reviewer will probably find most occasion to refer to the data in the chapters on wind, radiation, temperature, precipitation, the climates of coasts and inland water bodies, of soils, slopes and vegetation, and the climates of towns. But nearly every chapter is packed with data, presented in forms likely to be of wide usefulness, and some of the other chapters also (for example, on synoptic climatology) are very good and up-to-date.

A few samples of items that struck one reader as interesting either *per se* or as indicating lines of development of the subject will illustrate the content and flavour of the book. First, a point that indicates *inter alia* where further development is needed: the presentation (pp62–68) in text, map and tables of extreme windspeeds having average recurrence periods of just once in 50 and once in 100 yr, derived by sound statistical theory and adopted by the British Standards Institution for basic design windspeeds for buildings, are based in many cases on only 20–30 yr of record (and, in at least some cases, it may be presumed based on the very unrepresentative decades earlier in this century). The table of average yearly number of days with gale at 19 sample places illustrates not only the real gross geography of gales in these islands but how localities (and other things) may change with time and affect the issue. The second lowest frequency of gales (1.3 days per year) is at Mildenhall, Suffolk, now on the edge of Thetford Forest, an area which was known in the seventeenth century when it was open barren land for its storms of blowing sand that ruined many estates.

It is odd that there is no reference to hail in the index, but in the precipitation chapter we learn (p129) that this is an item which has been insufficiently studied and for which data are sparse. Many readers will be interested in the opportunity to compare the raininess of different places in terms of the average duration of rainfall: within the British Isles (in the 1950s) this ranged from $407\,\mathrm{h\,yr^{-1}}$ at Ipswich (427 in the London area) to 616 at Plymouth, 1,157 at Swansea and up to 1,422 in the Western Highlands of Scotland. Cloud-cover apparently varies much less widely, from an overall average of 6.7 tenths at Aberdeen and 7.0 in London and East Anglia to 7.8 in the west and north of Scotland. Another aspect of the western climates comes out in the temperature chapter, where the extreme south-west, the coasts of Wales and south-west Ireland, so favoured for retirement, are pointed up as the areas where the average winter temperatures are not low enough for the vegetation (and the grass of your lawn!) to cease to grow.

Other interesting features include a map of the summer albedo of England and Wales, and diagrams detailing the radiation falling hour by hour on slopes of various angles facing north, east and south. A map of the frequencies of fog, with the greatest frequencies (up to 60–70%) occurring on the high ground, reminds us how poorly represented are the hills in this country by observing sites since the discontinuation already in 1904 of the pioneer Ben Nevis Observatory. There is much that is interesting also in the chapter on coasts and inland waters, although we learn that most of the study of the climate of the latter so far has been left to the freshwater biologists.

This book is a worthy successor to Bilham's, keeping abreast of the climate itself and our developing knowledge of it and its impacts. Altogether good value at the price. ☐

H. H. Lamb is Director of the Climatic Research Unit at the University of East Anglia, Norwich, UK.

Engineering geology

J. L. Knill

Principles of Engineering Geology. By P. B. Attewell and I. W. Farmer. Pp. xxx + 1045. (Chapman and Hall: London; Halsted/Wiley: New York, 1976.) £25.

ENGINEERING GEOLOGY has been the main growth subject within the industrially-orientated aspects of the Earth sciences during the past two decades. The question remains, however, as to whether the success of engineering geology has been a consequence of the development of a new discipline, or is more fairly a reflection of the vocational opportunities for geologists within the construction industry.

The origins of engineering geology, as it is now defined, lay in the heavy construction industry in the early years of this century when geologists were invited to advise firstly on ground problems encountered during construction, and subsequently to assist in the prediction of difficulties during the earlier phases of investigation and design. At that time soil and rock mechanics did not exist as such and so it was natural in civil engineering for geologists to continue to be employed in the United States for the following decades; it was not until the 1950s that systematic education and growth in employment opportunities began to develop on a world-wide scale.

Soil mechanics was, by then, a well-established branch of engineering science, and subjects such as rock mechanics and hydrogeology were also firmly established. Perhaps it was inevitable that engineering geology has tended to move into a central position within the engineering earth sciences, attempting to link together the interactions between rock, soil and water as engineering materials within the realities of natural geological conditions and engineering problem-solving during investigation, design and construction.

There is, in consequence, a considerable element of choice not only in the balance between the component subject areas but also in the extent to which these subject areas are integrated with one another. On the one hand, it can be argued that the professional engineering geologist is essentially a geologist and his knowledge of the engineering disciplines should be maintained at a level that enables him to be able to communicate effectively in an engineering environment. On the other hand, engineering geology can be viewed as representing a balanced approach towards geotechnical engineering drawing together soil and rock mechanics, hydrogeology and geology into a unified whole to that the engineering geologist adopts the role of an engineer.

The authors of this textbook have been concerned for some years with the organisation of a postgraduate course in engineering geology at Durham University, and the structure of the book reflects both the philosophy of this course, and the associated research activities. The book falls into two broad sections, the earlier part providing an approach towards the study of the engineering properties of engineering soils and rocks, whereas the later part deals with more practical, engineering-orientated topics.

The opening chapter provides a general coverage of minerals and rocks, drawing particular attention to clay minerals and their identification and this is followed by a concise review of soil mechanics. The succeeding chapter is a major treatment of the geotechnical and geological characteristics of clays and clay shales, much of the relevant material having been drawn from unpublished research. The text turns to rock as a material, and the influence of symmetry and preferred orientation of micro- and macrofabrics on the properties of rocks and soils. The progressive approach of the authors towards the development of macroscopic fabric diagrams related to stability/instability fields is of particular interest.

At this point the book seems to change step, turning systematically to site investigation, groundwater, soil and rock slope stability, techniques of ground improvement, and the assessment and control of water resources. Most surprisingly, the application of geology to underground works and foundations, two important facets of the subject, are almost completely ignored.

This textbook is an interesting view of engineering geology, more strongly reflecting the engineering aspects than the geological. The arrangement of the book into two parts with somewhat different themes, is, however, not wholly satisfying nor has there been adequate control of excessive detail which penetrates into the text. For a book in excess of a thousand pages and at a price that will deter many, more careful structuring of the book with regard to the potential market could have yielded a shorter, cheaper and more comprehensive text.

The publishers suggest that the book will be of value for courses in both civil engineering and geology; at this price it can only possibly attract those who have already chosen engineering geology or geotechnical engineering as their career field. It has also been suggested by the publishers that "the book will itself eventually become synonomous with the subject area of engineering geology". Many will debate this prediction but it does not detract from the valuable service that the authors have done in drawing together so much useful material. ☐

J. L. Knill is Professor of Engineering Geology at Imperial College, University of London, UK.

Dating in archaeology

Elizabeth Slater

Dating in Archaeology: A Guide to Scientific Techniques. By S. Fleming. Pp. 272. (Dent: London, 1976.) £8.95.

A DECADE AGO, when the application of scientific dating techniques to archaeological material was gaining momentum, this book would have been a welcome addition to the bookshelves of archaeologists. Non-specialists then needed a general introduction to the available methods, with some indication of their potential and limitation, to illustrate the wealth of information that could be extracted from archaeological material and to assist them in the assessment of the validity of the dates produced.

This book follows a standard format with sections devoted to the main dating methods, giving information on the method, the materials to which it can be applied, its accuracy, associated problems and selected examples of its application. There are eight main chapters, plus a useful introductory chapter on the chronological patterns that were developed before the main impact of scientific dating methods, and a final chapter on scientific techniques applied to art history. The methods which reveal aspects of climatic changes are considered in an appendix and the other appendix contains an outline of the pre-dose thermoluminescence technique.

One of the greatest problems facing archaeologists is the interpretation of the information yielded by scientific dating methods. They are left with dates, plus associated errors, and a bewildering array of methods for the manipulation of the data. This problem is more readily acknowledged in the case of radiocarbon dating but it is inherent in all dating methods. The author gives no guidance in this matter, nor does he mention all possible procedures. He may feel that it would be premature to do so, and, as he says, "So many of the finer details of radiocarbon variations remain unresolved that no strong attempt has yet been made to routinely correct older conventional dates . . .". But he adds: "the reader may be tempted to adopt at least one form of correction factor to produce more absolute ages . . ." (p73). The non-specialist, who has to make a selection and evaluate conclusions, understandably requires assistance, or positive or negative recommendations. In the examples given of the application of the methods, Dr Fleming concentrates on the results, and not the validity of their interpretation.

The selection of examples of applications is wide-ranging, and one of the merits of the book is that it draws on material not readily accessible, but some omissions are rather surprising from an archaeological viewpoint. For example, scientific dating has had a considerable impact on the chronology of prehistoric Europe, but two references to the same paper is scant recognition of the lengthy debate which has produced a book and numerous papers. It may be considered that no conclusions are yet possible or that the topic has already been fully discussed, but readers familiar with the arguments will also be conversant with much work in this book. Also, the chronological framework discussed in chapter 1, and that indirectly derived from it, have been discussed in the light of new dating evidence, and a final summary of the continuing validity of the original patterns would have been helpful.

The topics discussed are continually being developed and modified, and the author has made a conscious effort to keep the bibliography up-to-date. Although it is useful to find descriptions of methods, like acid racemisation, which have so far only achieved limited recognition, from the point of view of archaeologists the changes in the main methods have been evolutionary rather than revolutionary in the few years to elapse since the last synopsis was published.

Despite the comments above, there is much of merit in this book. It contains a wealth of information, it is up-to-date, the methods are described clearly with an adequate amount of detail, and the diagrams are particularly clear. One distinctive feature is that analytical techniques and applications which have chronological implications are rightly included. It can certainly be recommended as the most up-to-date text for non-specialists, and the author is only unfortunate that he has followed a path which a number have trod. Perhaps the definitive book on dating, which Dr Fleming is admirably qualified to produce, is not destined to appear in the immediate future. ☐

Elizabeth Slater is Lecturer in Archaeological Science at the University of Glasgow, UK.

History of the Nile valley

Gudrun Corvinus

Prehistory of the Nile Valley. By F. Wendorf and R. Schild. Pp. xxiv+404. (Academic: New York and London, 1976.) $25; £13.75.

THE INTENTION of this book is to assemble and present all the available data and observations made during three seasons of fieldwork (1967–69) along the Nile in Egypt. The first part of the book covers in considerable detail the geology and stratigraphy of the Final Pleistocene Nile sediments between the Assuan and Fayum Depressions, including comprehensive descriptions of sites and sections. The second part describes and revises the sequence of stratigraphical and chronological events, and attributes the archaeological sites to cultural units.

The book is well illustrated with maps, section drawings, drawings of artefacts and photographs which clarify the rather lengthy descriptions of the sites. Regrettably a few names of the areas discussed are not mentioned in any of the maps.

The title of the book is somewhat misleading in view of the detail concerning the geology and palaeoenvironment; this latter is, nevertheless, of considerable value. The purely archaeological descriptions occupy only a small portion of the book.

The most significant contribution that the book makes is the revision of the sequence of events of the Final Pleistocene and Early Recent periods. The authors have assembled and interpreted a considerable amount of new data, filling gaps in the existing knowledge of the prehistory of the Nile and leading them to revise previous concepts.

The principal amendments concern the two large aggradations of the modern Nile. The Ballana Masmas episode is regarded here as one unit of simultaneous dune formation and Nile silt aggradation. A number of carbon-14 dates place this period between 17,000 and 15,000 BC, two different archaeological complexes having been recognised (the Idfuan and the Fakhurian). Furthermore, the Darau Member of the Kom Ombo area and the late Sahaba Formation of Sudanese Nubia belong, according to the authors, to the same episode of Nile aggradation and are termed here the Sahaba–Darau aggradation. The archaeological sites attributed to this period, dated from 12,000 to 9,700 BC, are the Afian, the Sebilian and the Isnan industries.

The authors are to be commended in attempting to compile and interpret such a vast amount of data. The book will serve as an excellent base for further research and as a reference for comparative studies, and will mainly interest the specialist in this field. ☐

Gudrun Corvinus is a consulting archaeologist to the Consolidated Diamond Mines of South-west Africa, Oranjemund.

Lake Rudolf Basin

G. E. Kennedy

Earliest Man and Environments in the Lake Rudolf Basin: Stratigraphy, Paleoecology and Evolution. Edited by Y. Coppens, F. Howell, I. Clark, L. Glynn and R. E. F. Leakey. Pp.xxi+615. (University of Chicago: Chicago and London, 1976.) Cloth £11.90; paper £5.80.

PALAEOANTHROPOLOGISTS have often discussed the value of an interdisciplinary, 'synthetic' methodology and yet this approach has not been highly visible in the literature. This book, and the recently published *After the Australopithecines* (ed. K. W. Butzer and G. L. Isaac; Mouton: The Hague and Paris; Aldine: Chicago; for review, *see Nature*, **262,** 331, 1976) may represent a strong sign that broad interdisciplinary analyses of early man sites will become the rule rather than a rarity. It is no doubt a sign of this trend that reports of the hominids found at East Rudolf comprise only five papers out of a total of fifty in this volume. These papers, presented at a workshop held in Nairobi in September, 1973, have been arranged into three sections: Geology and Geochronology; Paleontology and Paleoecology; and Paleoanthropology.

From this volume it is apparent that there are areas of both agreement and disagreement among the thirty-eight scientists who were involved in the Nairobi workshop. An interesting area of agreement concerns the occurrence of a climatic change between 2.5 and 2.0 Myr BP. This change, which resulted in drier conditions and an expansion of grassland areas in the Rudolf basin, has been indicated by studies on pollen (Bonnefille), bovids (Gentry) and micromammals (Jaeger and Wesselman). Documentation of climatic change at this time is of particular interest because the first clear evidence of archaeological materials also occurs within this period. Although the relationship, if any, between climatic change and the appearance of cultural materials is unclear, this interesting association requires more investigation.

From the standpoint of early man studies, one of the major outstanding questions is the date of the KBS tuff. The date of this tuff is crucial because there is good evidence of the presence of the genus *Homo* below this level. The results of radiometric studies have been contradictory, ranging from 1.6 to 2.6 Myr BP for this tuff. As Cooke's paper indicates, the earlier date for the KBS tuff may be suspect on palaeontological grounds. Cooke's studies on fossil pigs has shown that tooth size in one taxon, *Mesochoerus limnetes*, found below the KBS tuff at East Rudolf most closely resembles that in specimens from the nearby Omo area dated to 2 Myr BP.

This important volume contains a wealth of detailed and important information, and will be very useful at the professional level. Its information, however, will be less accessible to students. Although each of the three major sections is prefaced with an introduction, these are far too brief to really open the major issues and questions posed by each area of research. Such interdisciplinary studies are most valuable when they result in a synthesised and comprehensive view. Although such synthesis is missing here it is a minor defect in an otherwise fine volume. ☐

G. E. Kennedy is Assistant Professor in the Department of Anthropology at the University of California, Los Angeles, California.

Hominid and the bathwater

W. W. Bishop

Geology of the Olduvai Gorge: A Study of Sedimentation in a Semiarid Basin. By R. L. Hay. Pp. xvi + 203. (University of California: Berkeley, Los Angeles and London, 1976.) £15.75.

RECENT interest in Earth science has promoted fruitful growth of such 'interface' studies as geochemistry, geophysics, and geobiology. It comes as no surprise to see the common ground between geology and archaeology also attracting attention. What is unusual is to find, within the 200 pages of a single-author volume, a blue-print for the application of various disciplines to the study of sediments yielding hominids and artefacts. These include stratigraphy, geochronology, sedimentary petrography, mineralogy, isotope geochemistry and amino acid chemistry, to mention but a few.

On this substratum Dr Hay builds a sophisticated time framework for the past 2 Myr and reconstructs palaeoenvironmental settings for the hominids, their artefacts and, by inference, their activities in the changing palaeogeography of the Olduvai region.

Olduvai was the area where large scale excavations by Louis and Mary Leakey showed geologists, vertebrate palaeontologists and even some archaeologists, how local microstratigraphy could be studied to set early hominids and their palaeolithic took kits against a factual background from which to decipher the environment of burial and, with luck, the habitat of life. Dr Hay, with early experience of the stratigraphical complexity posed by arid intermontane basins in western USA, was the ideal successor to other geologists (Hans Reck, E. J. Wayland, P. J. H. Boswell, P. E. Kent and R. A. Pickering) who worked at Olduvai. It was fortunate that he appeared on the scene in 1962 after initial over-concern with alleged pluvial–interpluvial 'climatic stratigraphy' had been passed over in favour of a return to 'normal' stratigraphical and geological methods.

The finding of the skull of *Australopithecus* (*Zinjanthropus*) *boisei* in 1959 was the trigger mechanism for new investigations. Olduvai witnessed the first application of K–Ar dating to rocks as young as 1 or 2 Myr. Dr Hay tabulates the 57 K–Ar ages established for Bed I since the initial investigation by Curtis and Evernden, and discusses them in relation to fission-track dating and palaeomagnetic polarity data. Beds II to IV yield few reliable K–Ar dates, but numerous radiocarbon ages are given spanning the past 30,000 yr at Olduvai.

For the archaeologist the most important sections of the book are those presenting palaeogeographic reconstructions, both descriptive and visual (18 out of 27 maps in a total of 63 text figures), based upon detailed stratigraphical columns, which are also excellently illustrated by pull-out sections. These provide the factual and the interpretative environmental record against which Dr Hay records the hominid and artefact content of each formation. The final chapters present a synthesis in the form of a four-page evolutionary history of the Olduvai basin and a discussion which in 7 pages distils evidence for the pattern of hominid activities.

Despite its high price this is a book to purchase as well as to read, for its seems certain to become a 'classic'. It demonstrates how geochronology and the study of palaeoenvironments may be used to obtain full value out of both the fossil baby and the sedimentary bathwater.

W. W. Bishop was Professor of Geology at Queen Mary College, University of London, UK. He died on 19 February this year at the age of 46.

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Development of bioenergetics

A. R. Crofts

A New Look at Mechanisms in Bioenergetics. By E. Racker. Pp. xiv+197. (Academic: New York, San Francisco and London, 1976.) $9.50; £5.80.

PROFESSOR RACKER has ranged widely in his experience of research in bioenergetics, and this small book chronicles his activities in the field over the past ten years or so. The book takes the form of, and is based on, a series of lectures to students; it is aimed at the undergraduate or early postgraduate level rather than at established research workers. The chapters are prefaced by some admirably chosen quotations, and the text is interspersed with homilies and cautionary tales. The subject matter covered includes substrate level and electron transport-linked phosphorylation, ATP-driven ion pumps, resolution and reconstitution of the different complexes of enzymes which catalyse these activities, and details of the enzymology of these complexes.

Racker has played a major role in catalysing an acceptance of the chemiosmotic view of the mechanism of catalysing an acceptance of the chemiosmotic view of the mechanism of coupling between electron transport and phosphorylation. The book describes fully the very nice work carried out by Kagawa, Hinkle, Kandrach, Ragan and many other colleagues in collaboration with Racker, on the reconstitution of oxidative phosphorylation in liposome vesicles, using the purified enzyme complexes of the mitochondrial apparatus. This work had a strong biochemical appeal, which won over many of those in the field (and there were many) who had blanched at the large body of biophysical evidence previously available, that supported a similar view. The demonstrations that the mitochondrial ATPase, cytochrome oxidase and NADH$_2$ dehydrogenase could separately act as proton pumps when incorporated into liposome vesicles, and that when the ATPase was incorporated together with any of the electron transport complexes, the electron transport could drive phosphorylation, provided a powerful support for the argument for a chemiosmotic mechanism.

Most dramatic of all, however, was the demonstration, in work with Stoeckenius, that the purple membrane protein of Halobacter could be similarly reconstituted as a light-driven proton pump, and that inclusion of the ATPase in the vesicles led to a preparation capable of photophosphorylation. In this case, a clear distinction was evident between the requirement for a device for pumping protons, and an electron transport system. The former was necessary, but not the latter, so that the obligatory coupling between electron transport and phosphorylation predicted by chemical and conformational hypotheses for the mechanism of coupling could be excluded. It is perhaps a minor criticism of this book that the controversy over the mechanism of coupling is framed in such a way as to highlight these biochemical contributions. This is only human; the book cannot be recommended as a completely balanced view of the development of the subject, but, rather, as a personal view.

The other criticism I have to make relates to the "lessons" which crop up here and there, especially at the beginning of the book. These are asides, included perhaps to entertain the reader, and for the most part they are sensible and amusing. Some of them, however, strike me as ham-fisted. For example, Racker is being both cynical and unjust when he teaches with reference to Mitchell's preliminary paper on chemiosmotic coupling: "If you propose a brilliant, new hypothesis and avoid being specific, you probably will be ignored. If you want attention be sure to include a few features that are not essential for the main theme, but open to attack". Several other of the lessons also read like tips to a trainee con-man rather than distillations of wit and wisdom. The quotation above reflects more Racker's own late conversion to Mitchell's point of view than the considerable impact of this early paper; Racker (to his credit) draws attention to this tardiness of his in the preface.

These criticisms are, however, trivial, and I would not wish to discourage anyone from reading this book. It provides a lively and fresh insight into the subject, avoids obscurity and mystification, and provides a more than usually straightforward account of the development of an area of research, mistakes and all, in one of the most influential laboratories in the field. □

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Molecules in solution

J. M. Creeth

Biological Macromolecules and Polyelectrolytes in Solution. By Henryk Eisenberg. Pp. xviii+269. (Clarendon: Oxford; Oxford University: London, 1976.) £16.

FEW developments in physical biochemistry over the past two decades have had greater impact than that initiated by Casassa and Eisenberg in the years 1960–64. In a notable series of papers, a theory was described which dramatically simplified the measurement of molecular weight in ternary systems. This was most timely: it coincided with a surge of interest in charged polymers and with the recognition of the subunit structure of many proteins, where measurements were often necessarily made in high concentrations of urea, etc. Such systems were now shown to be amenable to analysis by pseudo-ideal equations, with the twin provisos that the solutions were at dialysis equilibrium and, most characteristically, that the multiplying coefficients (for example, a specific density increment) were defined at constant chemical potentials of the permeating components. In this way, all the effects of interaction (no matter how great) disappeared with the introduction of the appropriate coefficient.

The theory was very soon accepted, but, by the majority, perhaps, more on trust than by conviction, for the mathematics were undeniably tedious. The appearance of a volume by Professor Eisenberg devoted to a comprehensive account of macromolecules in solution is therefore an important occasion. The author has taken full advantage of the opportunities presented to him: a useful summary of the chief structural features of proteins and nucleic acids is followed directly by chapters on the fundamental thermodynamics of ternary solutions. Here the Casassa–Eisenberg concept is developed from first principles: this section contains a clear exposition of the meaning of the preferential interaction parameter, which should be read carefully by anyone tempted to refer loosely to 'solvation' or 'salt-binding'. The notation has been considerably simplified, and accordingly the essential concepts can now be grasped much more readily.

In the chapter devoted to partial volumes, density and refraction increments, the significance of the restriction of constant chemical potential is fully explained: a simple sketch brings home forcibly the meaning of each step. Here also we find the essential conclusions concerning the magnitude of the interaction coefficients: the practical demonstration that these do not vanish in the limit of zero concentration is, of course, the justification of the method. Most readers, I think, would now agree that the conventional 'buoyancy factor' in the equations of sedimentation should be replaced by the density increment, although some would, perhaps, regret the loss of the conceptual aid provided by **v**, with its reminder that the experiment determines the molar volume.

The remainder of the book is devoted to the four chief experimental procedures—light scattering, sedimentation equilibrium, sedimentation velocity/diffusion, and viscosity—each being treated in terms of the new definitions when appropriate. In addition to conventional methods, the newer technique of intensity-fluctuation spectroscopy is described, and the chapter on sedimentation equilibrium includes a section on density gradients which is notable for its vindication of the original theory of Vinograd and colleagues for the distribution of charged macrospecies. Those wishing to find their way through the tortuous paths of associating systems will find the rather sweeping assumptions made in this field clearly set out and assessed, and a useful summary is given of the procedures devised by Yphantis and Roark for the elimination of the effects of non-ideality.

The equations of transport are distinctly more complex than those relating to equilibrium, but this section will interest many for its revision of the theory of Alexandrowicz and Daniel, and the subsequent proof that the limiting sedimentation coefficient of a charged polymer may also be described in terms of the macromolecular component alone, when this is formulated in terms of the theory.

The volume is completed by a series of appendices devoted to thermodynamic concepts (including irreversible process theory) and the references, which are conveniently divided into categories of broad interest and of specific detail. The general standard of presentation is excellent, the few printer's errors noticed being essentially trivial—no mean feat in view of the complexity of notation.

The book will undoubtedly please the theoretician, for the logic and elegance of the mathematics; however, I think that most of all, it will be a delight to the concerned experimentalist, who will find the essential practical procedures and necessary precautions summarised concisely, in readily accessible form, together with the underlying theory. No laboratory working with macromolecules in solution should be without its copy. ☐

J. M. Creeth is Research Fellow in the Department of Biochemistry, University of Bristol Medical School, Bristol, UK.

Darwinism at a cellular level

Leslie Brent

Immunology, Aging and Cancer: Medical Aspects of Mutation and Selection. By F. M. Burnet. Pp. 162. (Freeman: San Francisco, 1976.) Hardcover $9.95; softcover $6.50.

THIS slender volume is based on lectures given by Sir Macfarlane Burnet in 1975 to students at the University of California, Irvine, and at Southampton University Medical School. It is an audacious and successful attempt to scan the wider horizons of immunobiology and genetics by examining and linking such fundamental issues as immunological homeostasis, the ability of cells to repair errors created in their DNA during the mutagenic process, the problem of aging, the pathogenesis of certain genetic and autoimmune diseases, and cancer.

According to Burnet, the common thread of the book is Darwinism at a cellular level. Although other theories have been put forward, it is somatic mutation that is most likely to account for the great diversity of antibodies and the cells that produce or carry them, and it is somatic mutation—coupled with a diminishing effectiveness of DNA polymerases and other enzymes in aging organisms to repair DNA errors—that leads to the manifestations of old age. Likewise, it is the accumulation of DNA errors in lymphoid cells that results in autoimmune disease, whereas the same phenomenon in somatic cells in general leads to the formation of malignant clones that, clinically, we call cancer.

Burnet is as adventurous and vigorous as ever in his discussion of these problems. Although it is true that many of his ideas have been developed at greater length in his earlier writings, this does not in the least detract from the value of this book; on the contrary, it is immensely readable precisely because of the economy of argument. The combination of brevity and global approach nevertheless imposes its own limitations, examples and data are selected fastidiously and, despite occasional warnings that the author's notions may eventually be shown to be wrong, the reader is sometimes left in the dark about points that might have been regarded as detrimental to them. For example, the superficially attractive idea that mast cells are fully differentiated T lymphocytes, bearing roughly the same relationship to each other as plasma cells do to B lymphocytes, is itself based on a questionable premise—that the immunoglobulin receptors of T cells are secondarily acquired from B cells shedding superfluous monomeric IgM. The existence of immunoglobulin-like T-cell receptors, let alone their origin, is still disputed. If it is true that T cells do not possess home-made receptors and that B-cell receptors are created by somatic mutation, it is reasonable to ask how it is that T cells have eluded this basic genetic mechanism and why it is that these cytophilic receptors are so difficult to demonstrate on T cells.

In the chapters on mutation and aging, Burnet explains that both bacteria and mammalian fibroblasts can make good errors in their DNA caused by *in vitro* exposure to ionising radiation. He proposes that the differences in the average lifespans of mammalian species can be largely, if not wholly, explained by differences in the efficiency of the DNA repair mechanism. Apart from the fact that this hypothesis disregards other possible factors, such as the more severe metabolic requirements imposed on small mammals by their high surface/volume ratio, even experts in this field are not yet certain of the extent to which DNA repair influences lifespan. In the words of R. E. Hart (in *Aging Carcinogenesis, and Radiation Biology*, ed. K. C. Smith, Plenum: New York, 1976.): "Indeed, at this time it would be premature to infer that DNA repair in and of itself is a primary determinant of lifespan, much less to state that a single repair process is of more significance than any other". Be that as it may, Burnet does not envisage that immunological processes play a primary part in aging but, rather, that they are brought into play as a result of accumulated errors in aging somatic cells, thus producing autoimmune diseases; and that aging of the lymphoid cells themselves leads to decreased efficiency in immune surveillance, thus permitting the development of malignant clones. Little credence is given to the suggestion that viruses may be playing a critical role in some so-called autoimmune diseases, and in re-stating the hypothesis of immune surveillance there is no mention of the experimental evidence that has, in recent years, somewhat weakened this highly attractive idea—certainly so far as T-cell surveillance is concerned. I am thinking here mainly of the observations that neither "nude" (T-cell deficient) mice, nor mice treated from birth with antilymphocyte serum, show a markedly increased incidence of various kinds

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of tumours. That immunosuppressed kidney allograft recipients have a greatly increased incidence of lymphoid tumours provides far from convincing evidence for the hypothesis, as the immunosuppressive drugs used could themselves be carcinogenic for lymphocytes or, alternatively, chronic stimulation by alloantigens might act as a trigger for malignancy in lymphoid tissues.

We must be grateful to Sir Macfarlane for giving us a highly readable and stimulating book, although the general reader should be warned that some background knowledge of immunology or genetics is desirable if he is to get the most out of it. ☐

Leslie Brent is Professor of Immunology at the Wright–Fleming Institute, St Mary's Hospital Medical School, London, UK.

RNA polymerase

Walter F. Mangel

RNA Polymerase. Edited by R. Losick and M. Chamberlin. Pp. ix+899. (Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1976.) $38.

GENE EXPRESSION and its control has, for the past 30 years, been one of the more fruitful areas of research for molecular biologists. The intriguing results of the bacterial geneticists enticed many biochemists and physicists to seek molecular explanations for such widely diverse phenomena as the inducible metabolism of sugars, the initiation of DNA synthesis and the temporal expression of bacteriophage genes. In most cases, they ended up studying transcription. This book summarises their progress and illustrates the multifarious and subtle ways that have evolved to control gene expression at the level of RNA polymerase activity. Now they are studying the primary sequence of *lac* operator DNA, the RNA primers for DNA replication and the phage-induced RNA polymerases.

Losick and Chamberlain are to be congratulated for having designed an excellent book which will be useful to those who desire a comprehensive introduction to the subject, as well as to those who are actively carrying out research in the field. It is also an excellent text for graduate students. The first half of the book contains an historical introduction by Weiss, an overview of the transcription process by Chamberlin, and eleven review articles that outline the active areas of research. The second half consists of 32 previously unpublished research articles. As with all Cold Spring Harbor monographs, this one contains an extensive and useful list of references.

In his introduction, Weiss describes how the discoveries of different but specific tRNAs and of nucleoside triphosphates as substrates for DNA synthesis led him to assay for an RNA polymerase activity in a crude rat liver homogenate. And in the next article, Chamberlin summarises the work done in the intervening 17 years. His overview of both the molecular and catalytic properties of RNA polymerase is comprehensive, integrating properties of the *Escherichia coli* enzyme with those from other prokaryotic and eukaryotic cells. This is followed by a highly practical article by Burgess on the purification and physical properties of the enzyme. Zillig, Palm and Heil discuss the functions and re-assembly of the subunits, and Krakow, Rhodes and Jovin describe the use of inhibitors to study catalytic mechanisms. The intriguing problems of how RNA polymerase recognises a promoter, initiates transcription and terminates RNA synthesis at specific sites, are discussed in articles by Chamberlin, Gilbert and Roberts. Scaife delineates the progress in the location of the genes for RNA polymerase and the control of their expression. Transcription patterns in bacteriophage infections are controlled, in some cases, by regulatory subunits or bacteriophage-induced RNA polymerases, as outlined by Losick and Bautz. Roeder discusses the properties of nuclear RNA polymerases and some of the problems encountered in studying eukaryotic transcription. Finally, Kornberg defines the role of RNA primers in DNA replication.

The research articles in the second half of the book are specific extensions of each of the areas described in the first half. There are three articles on promoter sequences. With nine promoters having been sequenced, common features, such as twofold axes of symmetry, are emerging. RNA polymerase will not, however, rebind to promoter fragments isolated by protection from DNase, so that additional elements remain to be discovered. The inhibitor, rifampicin, has been extremely useful in studying RNA chain initiation, and there are three articles which indicate that the mode of action of the drug is surprisingly complex. Rifampicin-resistant mutants made possible the mapping of the β subunit. In four articles, the locations of the other subunits are described and shown to be linked with the genes of some of the proteins of the translation apparatus. There are four papers on the regulatory subunits which become associated with RNA polymerase after bacteriophage infection. With the discovery that the rho gene maps at the suA locus, the termination step in RNA synthesis is a very active area of study and is represented by six papers.

Finally, there are some excellent articles on eukaryotic RNA polymerases from yeast, *Drosophila* and mammalian cells. Although the activity of RNA polymerase is modulated to control gene expression in prokaryotes, similar mechanisms have not yet been observed in eukaryotic cells. An elephant may still be a big *E. coli* but it is, ironically, less obvious when viewed at the level of transcription.

This is an excellent book on an important and fascinating subject. ☐

Walter F. Mangel is an Assistant Professor of Biochemistry at the University of Illinois, Urbana, Illinois.

Genetic evolution

Thomas H. Jukes

Molecular Evolution. Edited by Francisco J. Ayala. Pp. x+277. (Sinauer Associates: Sunderland, Massachusetts; Freeman: Reading, 1976.) Paperback £6.90.

THIS book is a compilation of thirteen papers that were presented at a symposium held at the University of California, Davis, in June, 1975. Most of the articles are more closely related to population genetics than to molecular evolution.

There is an introductory chapter by Francisco J. Ayala followed by chapters on genetic variation in natural populations (Robert K. Selander); genetic polymorphism and enzyme function (George B. Johnson); allozyme variation: its determinants in space and time (Michael Soulé); genetic strategies of adaptation (James W. Valentine); organismic and molecular aspects of species formation (Theodosius Dobzhansky); genetic differentiation during speciation (John C. Avise); biochemical consequences of speciation in plants (Leslie D. Gottlieb); protein sequences in phylogeny (Morris Goodman); molecular evolutionary clocks (Walter M. Fitch); evolution of genome size (Ralph Hinegardner); evolution of repetitive and non-repetitive DNA (Glenn A. Galau, M. E. Chamberlin, B. R. Hough, R. J. Britten and E. H. Davidson); and gene regulation in evolution (Allan C. Wilson).

The discussions of molecular matters occur principally in the final five chapters. Goodman's observations lead to his concluding that the evolution of globin sequences in vertebrates proceeds at different rates during different evolutionary periods. He attributes the "fast evolution to positive natural selection for improvements in the function of hemoglobin and the slow evolution to stabilising natural selection after the improvements were fixed", following which "natural selection would then be able to shape the finer adaptations of the protein". It is possible to propose, however, various interpretations to account for such observations, and Goodman acknowledges this point.

Fitch, in discussing molecular evolutionary clocks, says that "we have several sloppy clocks", which may be an example of sloppy diction. He states that "the tRNAs are mostly paralogous", which was news to me, because there are many examples of homologous tRNAs for the same amino acid occurring in related species of organisms. Obviously, many thousands of such tRNAs have not yet been sequenced. Fitch tells us it came as something of a shock to him that Δ haemoglobin has never been sequenced, which leads me to comment that those who accept the sequences in the *Atlas of Protein Sequence and Structure*, instead of referring to the original literature from which the sequences are derived (sometimes by presuming the sequences of unordered peptides), should expect such shocks. Fitch gives a good discussion of the rates of 'silent' nucleotide substitutions. He challenges comparisons of snake, bird and turtle cytochromes *c* by suggesting that "either one or more of its sequences is incorrect", which is an easy out of the argument.

The review by Galau and co-workers is a valuable compilation of recent results on the rate of re-association of various types of DNA. Wilson presents an excellent summary of the differences in rates of evolution between frogs and placental mammals in support of his thesis that contrasts the rates of organismal change and structural gene evolution. The apparent similarities of different species of frogs to each other conceals the fact that they may be widely separated in terms of ancestral history, and also in terms of amino acid differences between their homologous proteins. Hinegardner discusses the wide variations in haploid DNA contents per cell in various groups of organisms. The DNA content per cell has increased during the history of life on Earth, but mammals do not lead the parade.

I wish that the term "nonsense" for polypeptide chain-terminating codons, and the non-word "missense" for mutations that produce single amino acid changes (as used and defined by Ayala) could be relegated to the graveyard of discarded and superseded neologisms. But new words never seem to die, or even fade away. ☐

Thomas H. Jukes is Professor of Medical Physics in Residence at the University of California, Berkeley, California.

Somatic cell hybrids

R. T. Johnson

Cell Hybrids. By N. R. Ringertz and R. E. Savage. Pp. 366. (Academic: New York and London, 1976). $29.50; £20.95.

THERE are relatively few events in the recent history of biology which have so fired and re-directed the imagination as somatic cell fusion. Suddenly, in the mid-1960s, the unexpected and unnatural seemed commonplace in cell biology. Somatic cells of diverse origin and nature could be brought together within a single cell, their interactions studied and their hybrid progeny cultured for subsequent analysis. The revolution that Sendai virus-promoted fusion brought to the embryonic subject of cell hybridisation not only permitted the achievement of a greater number of fusion events by a greater number of people but also generated the enthusiasm to develop hybrids between seemingly impossible combinations of cells. The search for agents that promote fusion between unlikely partners remains an area of continuing interest, with the recent demonstration of polyethylene glycol-induced fusion of human and plant cells.

The unusual attraction and usefulness of cell hybrids to biologists soon became evident from the growing number of publications covering a very wide area of contemporary biology. Cell fusion rapidly became one of the most attractive ways of detecting differences between cells. By combining two cells it is possible to monitor some of their differences by following interplay with respect to such general genetic activities as the initiation of RNA and DNA synthesis, and more specifically, to the extinction, re-expression and activation of single genes. Although fraught with difficulties, the analysis of the differentiated state by means of cell hybrids has now progressed to a stage where general rules for the expression of facultative functions can be expected before long. Similarly, the usefulness of somatic cell hybrids in gene complementation and mapping is in no doubt, and advances here and in the study of differentiated expression in hybrid cells are fortunate to have coincided with a revolution in methods for chromosome identification. Cell fusion has also provided an opportunity to study the nucleocytoplasmic interactions in mammalian cells in ways which are similar to the investigations so elegantly carried out using the technique of nuclear transplantation in large cells like Protozoa or amphibian oocytes.

In this well illustrated volume the authors have gathered together, for the first time, the accumulated work up to the end of 1975 on somatic cell fusion and cell hybrids. This is a large, complex, controversial and expanding field and we are therefore indebted to Drs Ringertz and Savage for this timely compilation and review of the subject, reduced to manageable proportions and written with clarity.

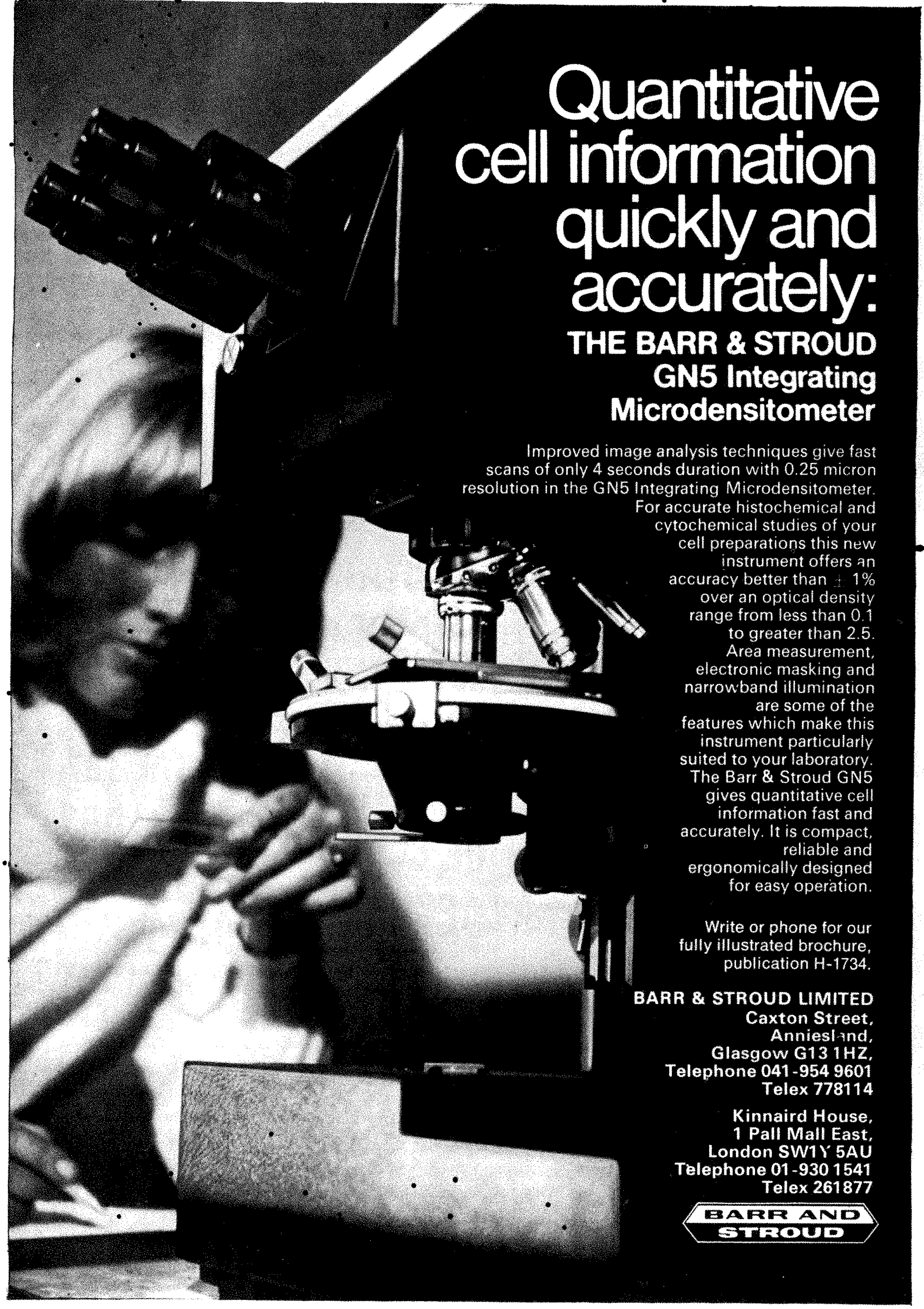
The aim of *Cell Hybrids* is to provide an up-to-date account of the contributions made by cell fusion and cell hybrid studies to major areas of somatic cell investigation including cell cycle control, the expression of differentiated functions and malignancy, and the mapping of constitutive genes. It is also intended that the volume should serve as an introduction to students and to workers in other areas. A useful background is therefore included in each chapter providing sufficient information for critical appraisal of results obtained.

Because of their limited lifespan animal cell heterokaryons are less frequently investigated than their hybrid progeny. As the authors stress, however, heterokaryons permit analysis of regulatory phenomena in the presence of complete genomes and should be included whenever possible in any full description of gene expression in hybrid cells which usually contain a reduced and occasionally modified complement of chromosomes. Possibly the most famous heterokaryons contain chick erythrocyte nuclei which, as Henry Harris showed 12 years ago, are reactivated from the quiescent state. Much of the elegant work on erythrocyte activation has emerged from the Karolinska Institute and this is well represented in *Cell Hybrids*.

The very great problems that attend the unravelling of genetic interplay in somatic cell hybrids may to some extent be reduced by the use of reconstructed cells, and a full description of this new area of cell manipulation is included. For example, the production of hybrids by the fusion of whole cells with microcells containing very small quantities of DNA not only predetermines the segregation pattern but may speed up and ultimately simplify the analysis of gene localisation and regulation.

The advent of cell hybridisation has permitted many new experimental approaches to major and seemingly intractable biological problems. A number of the problems have now been reduced in stature and others re-defined because of cell hybrids. We should be grateful to the authors for this comprehensive and readable work on the subject.

R. T. Johnson is a Research Fellow of the Cancer Research Campaign, working in the Department of Zoology at the University of Cambridge, UK.



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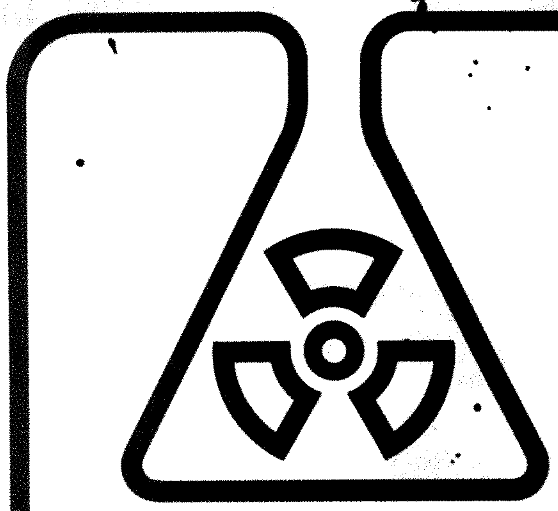

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articles

Interpretation of the Southern California uplift in terms of the dilatancy hypothesis

Max Wyss

Cooperative Institute for Research in Environmental Sciences, University of Colorado/NOAA, Boulder, Colorado 80309

The 25-cm uplift in Southern California is interpreted as being due to dilatancy of the crust, which started in 1961 along the San Andreas fault and spread laterally during 1964–69. The San Fernando earthquake of 1971 occurred in part of the dilatant volume, and is interpreted as a sideshow of a future repetition of the 1857 San Andreas break, which had a magnitude > 8.

AN area of at least 12,000 km^2 was uplifted aseismically in the early 1960s in Southern California[1]. The uplift was centred on the San Andreas Fault, where it reached a maximum of over 25 cm near Palmdale, 60 km north of Los Angeles. The portion of the San Andreas Fault that lies within the 15-cm uplift contour comprises the southern half of the last major earthquake in Southern California, the 1857 rupture, which had a length of about 350 km (ref. 2). The segment of the San Andreas Fault that broke in 1857 exhibited conspicuously low seismicity during the last 40 yr (ref. 3). This section is considered to be a mature seismic gap, which is expected to be closed by a large quake sometime in the future.

Castle *et al.*[1] offered two hypotheses to explain the portion of the aseismic uplift in the San Fernando area: (1) a north–south contractional strain effect coupled with creep on a deep part of the San Fernando Fault, or (2) a dilatancy effect. Thatcher[4], elaborating on the first hypothesis, proposed that the north-dipping San Fernando Fault reaches a depth of about 50 km, cutting below the San Andreas and extending north of it, and he suggested that aseismic creep started at the deepest part of this thrust fault and propagated upwards, causing the uplift and culminating in the 1971 San Fernando earthquake.

We formulate here a dilatancy hypothesis to explain the observed uplift and to show that this alternative has some advantages that may make it more plausible than the creep hypothesis.

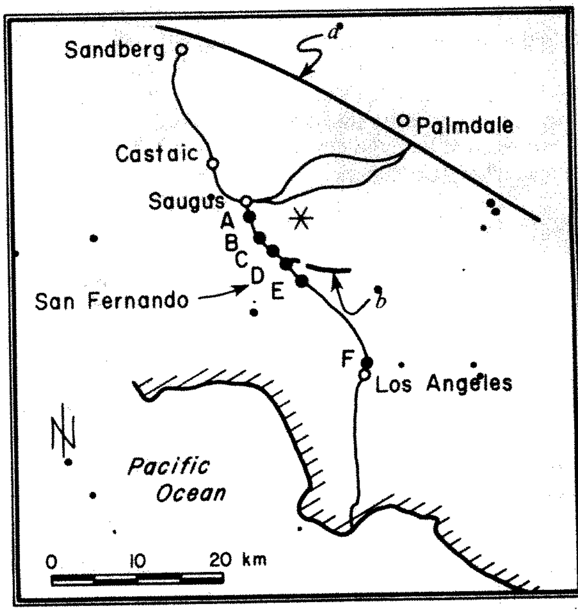
Uplift data

Figure 1 shows the location of the two levelling lines discussed by Castle *et al.*[1]: Los Angeles–Saugus–Palmdale, and Los Angeles–Saugus–Sandberg. Figure 2 shows the uplift as a function of time at the points A–F (Fig. 1), which straddle the 1971 San Fernando thrust fault (Fig. 1). The similarity of the uplift history before the San Fernando earthquake (Fig. 2) and the famous uplift before the Niigata earthquake[5] is striking. A 35–40-yr period of stability is followed by sudden uplift, which again is followed by a few years of quiescence. It is important to note that points A, B and C are located north of the San Fernando Fault on the upthrust block, and points D, E and F south of it on the downthrust block. All of these points were uplifted before the San Fernando earthquake. This feature is required by the dilatancy hypothesis, and it is opposite to what is expected by the creep hypothesis, as will be discussed below.

In Fig. 2 it is also evident that the uplift started earlier in the north (points A, B), and was larger there, than in the south (points E, F). This sequence is examined in more detail in Fig. 3, which shows the uplift along the Palmdale–Saugus–Los Angeles traverse. The picture is that of an uplift disturbance initiated in the north (at the San Andreas) and spreading south with the San Fernando earthquake as a preliminary culmination. A tiltmeter located anywhere along this traverse would have recorded a south tilt at first (1961–64), and then a north tilt (1964–65), as was noted by Thatcher[4] for the traverse Sandberg–Saugus.

The Sandberg–Saugus–Los Angeles traverse is shown in Fig. 4. For this traverse no data were available for the period 1961–64. During 1964–65, the uplift was concentrated in the middle to northern part of the traverse as it was for the other levelling line in the same period of time (Fig. 3). Next, the southern and northern portions were uplifted between 1965 and 1968. The middle portion stayed at the same elevation during this interval, but it caught up the next year, 1968–69 (Fig. 4*c*). The entire

Fig. 1 Map showing the location of levelling lines with respect to the San Andreas (*a*) and San Fernando (*b*) faults. *, 1971 epicentre. Uplift is measured with respect to the coastal point of the traverse (tidal 8) as constant[1].



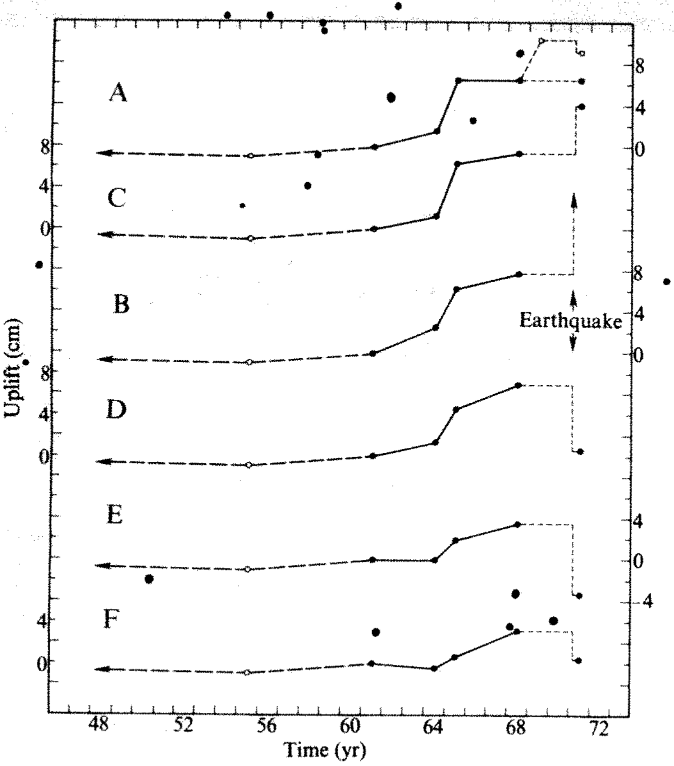


Fig. 2 Uplift as a function of time for three points each on the upthrust (A, B, C) and downthrust (D, E, F) blocks of the San Fernando quake. The data from 1961–71 are from Castle *et al.*[1]. Before that, two measurements in 1926 (→) and 1955 (○) were the same, within a couple of cm, as those in 1961 (R. O. Castle, personal communication).

southern part of the traverse may also have been uplifted a further substantial amount in 1968–69; however, there are no data along this portion of the surveying line. The possibility of this additional surge in 1968–69 is indicated in Fig. 2 for point A as alternative. Figure 4*d* shows the elevations along the traverse after the 1971 San Fernando earthquake.

Interpretation

From Figs 2–4 it is evident that uplift before the San Fernando earthquake occurred both north and south of its surface rupture, suggesting that dilatancy preceded this earthquake. Dilatancy is the volume increase exhibited by sands and rocks under large deviatoric stresses[6,7], is measured as the deviation of the volume change, $\Delta V/V$, from the linear elastic behaviour, and is due to increased pore and crack volume. In the laboratory, dilatancy precedes rock failure. It was proposed that dilatancy in the crust is the cause of observed changes of crustal properties before some shallow earthquakes[8–13].

If we assume that the entire uplift was due to dilatancy, we may calculate the volume change from[14]

$$\Delta V/V \approx h/r$$

where h is the uplift and r is half the depth to which dilatancy extended. I will assume that only the 'brittle' part of the crust (the top 15 km which contain earthquakes) can become dilatant[15]. Under this assumption, a 15-cm uplift between 1961 and 1964 near the San Andreas implies that $\Delta V/V = 2\times10^{-5}$, which is schematically indicated in Figs 3*a* and 4*a* by dark shading. The spreading of increased uplift away from the San Andreas towards San Fernando is interpreted as being due to an increased amount of dilatancy, spreading through the crust, away from the San Andreas (see crustal cross sections as a function of time in Figs 3 and 4). Finally, the San Fernando earthquake locally released the deviatoric stress, and dilatancy disappeared below the southern portion of the profile. The crust within 30 km of the San Andreas remained dilatant, in this interpretation, and the repetition of the 1857 earthquake will reduce this portion of the crust back to normal. This model also accounts for the tilt reversal, which was presented as one of the main supporting facts for the creep hypothesis[4], and is in agreement with laboratory observations[16], where localised bulges due to dilatancy are observed to migrate to different parts of the rock sample, and, in the final phase; begin to concentrate along the subsequent fracture plane.

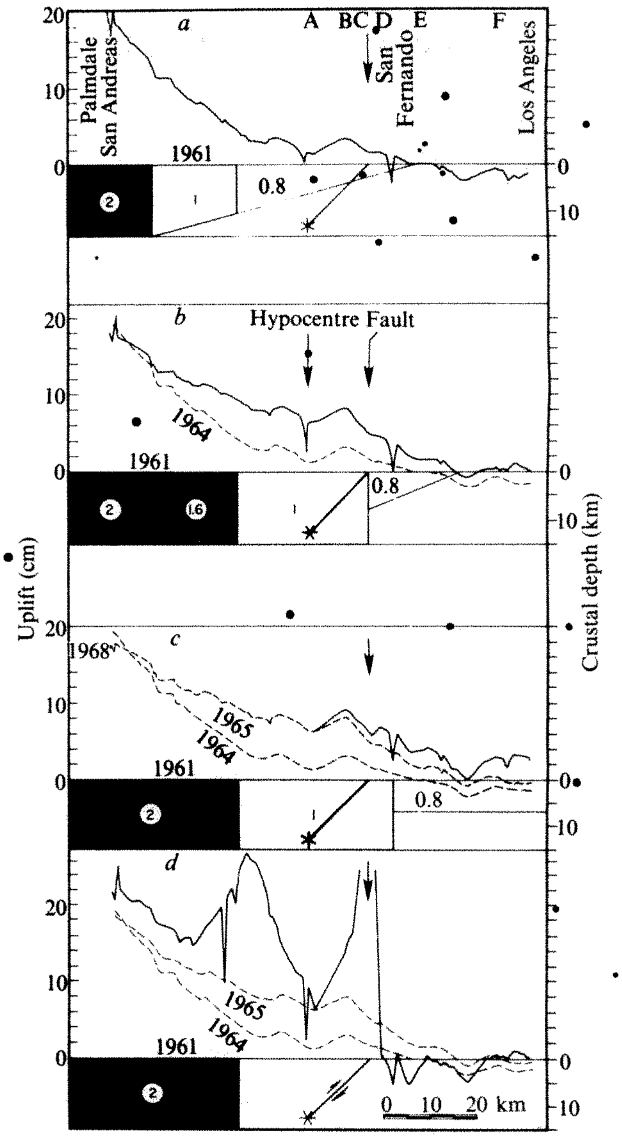


Fig. 3 Uplift along the Palmdale–Saugus–Los Angeles traverse from Castle *et al.*[1]. The 1961 level was taken as reference with respect to which the 1964 (*a*), 1965 (*b*), 1968 (*c*) and 1971 (*d*) levels are shown. The state of the crust below the traverse is shown in each time frame. The number in the crust indicates the volume increase in units of 10^{-5} if it is assumed that thickness of the dilated crust is that indicated by the stippled area. In the 1971 curve the hump north of the hypocentre is due to the corner in this profile which re-approaches the epicentre at this point. *a*, Uplift between 1961 and 1964, decreasing gradually from 20 cm in the north (at the San Andreas) to zero in the south (San Fernando). *b*, Uplift in 1965 (—) with respect to the 1961 level. The 1964 uplift is retained (- - -) for comparison. No further uplift occurred between 1964 and 1965 in the north, but a substantial amount was added in the south, near the future San Fernando rupture. *c*, Uplift in 1968, again being concentrated even further south than the 1964–65 episode. Finally, after the 1971 San Fernando earthquake (*d*), the previously uplifted area south of the rupture was now below the initial level, north of the rupture large co-seismic uplift occurred, and along the San Andreas the uplift was essentially unchanged.

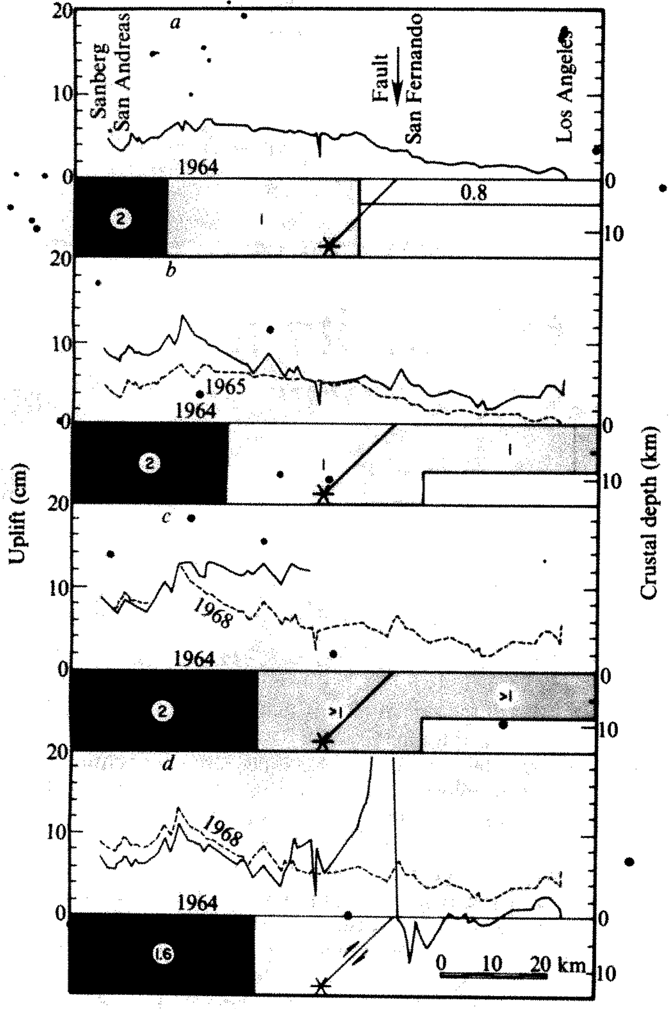


Fig. 4 Uplift along the Sandberg–Saugus–Los Angeles traverse from Castle *et al.*[1]. The reference level is that of 1964. Levels are shown for: *a*, 1965; *b*, 1968; *c*, 1969; *d*, 1971. Uplift episodes are again approximately interpreted by an increasingly dilated crust. The amount of volume increase is again given in units of 10^{-5} with the assumption that in the time interval 1961–64, for which no data were available, an uplift of 10 cm occurred near the San Andreas, decreasing to zero at San Fernando. The basis for this assumption was the uplift between 1961–64 on the other profile (Fig. 3), but divided by two so as to be on the safe side.

Why should dilatancy begin at the San Andreas, and why should it persist there at a time when rupture occurred in a less dilated volume? Geodetic measurements in California suggest that strain accumulation may be concentrated along the San Andreas Fault[17,22]. I will therefore assume that stresses are concentrated along the fault and hypothesise that the deviatoric stress *C′*, at which dilatancy appears[7], was first reached near the San Andreas. As further deviatoric stress was accumulating, *C′* was exceeded in a widening crustal volume. Also, as Thatcher[4] showed, the stress normal to the local trend of the San Andreas was increasing, thus locking the fault. The shear stress along thrust faults in the area increased, and so it follows that local thrust faults like the San Fernando Fault may fail first.

I also note that Thatcher[4] has shown that the surface stress was everywhere compressive perpendicular to the uplift contours. This observation is in agreement with the laboratory measurements on dilating samples. Nearly always the stresses on the outside of the buckling samples are compressive, that is, the samples continue to become shortened throughout the experiments up to failure, even though dilatancy occurs.

Another interesting point is that there were two surges of uplift, one in 1961–65 and the other in 1968–69. The latter may have occurred only in the middle part of the profile, including the epicentre region of the San Fernando earthquake. The second phase of uplift could have been due either to the pore fluid pressure increase expected as a corollary of the wet dilatancy hypothesis[18], or to precursory creep, as required by the dry dilatancy models.

Finally, the speed with which the uplift occurred might be taken to support the creep hypothesis. Laboratory results on jointed rocks[19] suggest, however, that in crustal conditions, dilatancy uplift of a few centimetres could occur within a few months. Several years before earthquakes in Peru, Japan and California, approximately 6 cm of aseismic uplift occurred which accumulated within less than half a year[20].

Conclusions

The Southern California uplift can be explained by a dilatancy model, as well as by a creep model. In fact, aseismic uplift, the only direct evidence for dilatancy, can always be explained alternatively by a creep model, unless gravity observations are available together with uplift data.

Thatcher's[4] creep model gives major importance to the transverse range. Its advantage is that it explains the observed asymmetry of the Southern California uplift (steeper to the south). Its disadvantages are that it does not explain the uplift south of the San Fernando rupture. The continuation of Thatcher's Fig. 4*b* to the south would have to show curves 1 and 2 below zero level, when the data show that this area was subject to uplift, not subsidence. Also, it does not explain why the uplift is centred on the San Andreas Fault, and it does not explain the Niigata data in a straightforward way.

The dilatancy model gives major importance to the San Andreas Fault as a plate boundary concentrating stresses. The transverse range follows from the bend in the San Andreas. This model's advantages are that it explains the entire uplift, including the uplift south of San Fernando. Also, it agrees with the interpretations of anomalous travel times[13] and anomalous seismicity[24] before the San Fernando earthquake. The disadvantage of this hypothesis is that it does not explain the asymmetry of the uplift. It is quite plausible, however, that the rheological contrast between the granitic transverse range and the deep sedimentary basin of Los Angeles may cause a different dilatancy response to the same overall stress.

A test for accepting or rejecting one of the models could be found in the seismic wave velocities during the early and mid 1960s. The dilatancy model requires that in the early stage a velocity decrease should have occurred. From 1973 on, no substantial velocity anomalies are observed in the general area of the uplift[21]. If ground water has returned to fill cracks and pores, however, the anomaly would have disappeared by now. Another test lies in the future developments in the area. The creep hypothesis was developed on the example of a very small feature, the 20 km long San Fernando rupture. If the creep hypothesis is correct, it should apply to the entire length of the uplift, which is at least 200 km long. The corollary is that ten more San Fernando-type thrusts should occur in the future along the transverse range front. If the dilatancy hypothesis is correct, medium-sized thrusts are not excluded, but the San Andreas would be expected to produce a major quake with some subsidence following it.

I feel that neither the creep nor the dilatancy hypothesis is strong enough to be accepted at this point. While the uplift south of San Fernando is best explained by dilatancy, it may be quite likely that premonitory creep occurred on a deeper part of the San Fernando fault, as required by the dry dilatancy models.

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Hydrothermal fluids of seawater salinity in ophiolitic sulphide ore deposits in Cyprus

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Freezing points of fluid inclusions in vein quartz intergrown with sulphides from ophiolitic cupriferous pyrite ore deposits in Cyprus indicate a hydrothermal fluid of seawater salinity, and confirm the hypothesis of metal transport in hot seawater. It is therefore suggested that the economic potential of oceanic crust layer 2 is probably negligible.

EXISTING information suggests that hot seawater leached, transported and deposited metals (Fe, Cu, Zn, Pb, Ni, Co, Ag and Au) from solution to form the sulphide ore deposits in the Upper Cretaceous ophiolitic rocks of the Troodos Complex, Cyprus[1–4]. The evidence is based principally on the use of isotopes (^{87}Sr/^{86}Sr, D/H, ^{18}O/^{16}O and ^{34}S/^{32}S) as geochemical tracers and is consistent with a model for hydrothermal metamorphism and mineralisation which involved convection of seawater within the ~2-km thick permeable layer of pillow lavas and dykes which forms the upper part of the ophiolitic sequence (Fig. 1)[1–5]. By estimation of a Rayleigh number Spooner[3] has shown that the fluid dynamic conditions necessary for vigorous hydrothermal convection were satisfied.

The ore deposits, which formed in the submarine environment, generally consist of a lens of massive sulphides (pyrite – chalcopyrite ± sphalerite ± marcasite ± galena) intercalated within the pillow lava sequence[6–10]. This is underlain by a pipe-shaped mineralised stockwork extending downwards into the dyke complex (Fig. 1)[6–10]. They have been worked for copper since pre-classical times, for high quality pyrite used in the manufacture of sulphuric acid[11] and for minor quantities of gold and silver, which occur in 'Devil's Mud' only, found in the zone of supergene alteration[6]. Approximately 20 deposits containing on average ~1 million tonnes ore of variable copper content have been worked on the island. The largest and richest was Mavrovouni, which contained ~15 million tonnes ore grading between 3.5 and 4.5 weight % Cu (ref. 6). Limni—the largest working mine at present—produces ~450,000 tonnes ore per annum at a mean grade of ~0.85% Cu (refs 12,13).

The object of this article is to describe a test of the hypothesis of metal transport in hot seawater. This has involved determination of depressions of the freezing point of samples of the ore fluid which occur as small inclusions in minerals from the sulphide deposits. This colligative property, a reflection of the salinity of the solution, is then compared with the freezing point of seawater.

Experimental technique

The fluid inclusions were examined in thin sections of rock a few millimetres in size which were polished on both sides down to a thickness of ~60 µm using 1–0.3-µm sized alumina particles on a vibratory polishing machine. The slivers were prepared from ~1-mm thick slices of rock containing minerals with fluid inclusions which were mounted on glass slides using Lakeside resin. The thin sections were floated off by soaking in absolute alcohol for 1–3 days, and pre-selected areas of inclusions were cut out. Microthermometric measurements were then conducted on a Chaix-Meca heating-freezing (−180 °C to +600 °C) stage[14] mounted on a Leitz Ortholux II microscope fitted with ×32 and ×50 UMK objective lenses and ×25 binocular eyepieces. This facilitated examination of inclusions down to a few micrometres in size. Measurements were carried out on about 20 inclusions per sample. The inclusions were pre-selected in order to obtain an approximately random sample.

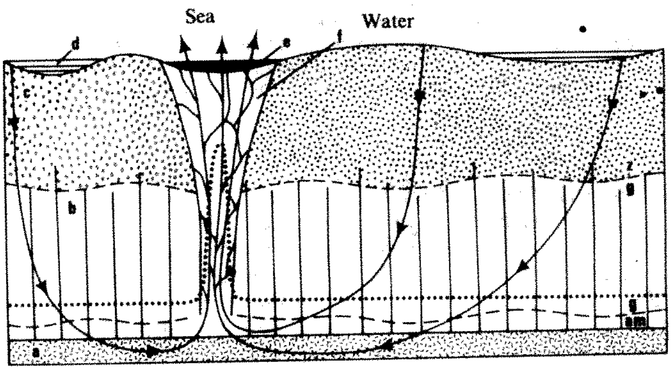


Fig. 1 Schematic diagram of model of hydrothermal convection, metamorphism and mass transfer within the ophiolitic rocks of the Troodos Massif, Cyprus. General metamorphism[37] occurred in zones of recharge flow, whereas formation of the cupriferous pyrite ore deposits[6–10] occurred in the discharge zones. Precipitation of Fe/Mn enriched sediments ('umbers')[38–40] was also probably a consequence of discharge of hot metalliferous solutions[1,38]. a, Gabbros, metagabbro and variably altered trondhjemite; b, sheeted dyke complex; c, pillow lavas; d, umbers; e, massive sulphide ore; f, mineralised stockwork. Dashed lines approximate to metamorphic boundaries between zeolite facies (z), greenschist facies (g) and amphibolite facies (am). Continuous lines are schematic flow lines for hydrothermal convection; dotted line is approximate form of an isotherm produced by convection.

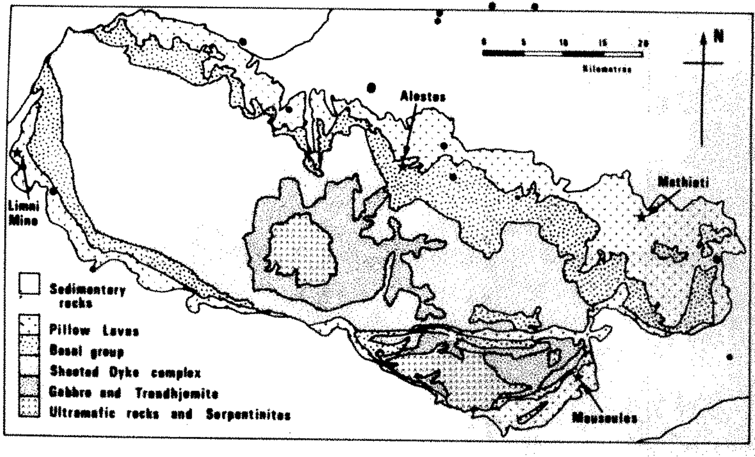


Fig. 2 Simplified geological map of the Troodos Massif, Cyprus (modified after the Cyprus Geological Survey map) showing the locations of the mines from which samples were obtained for fluid inclusion and strontium isotopic studies[21].

Pure nitrogen gas, which was passed through a heat exchanger in liquid nitrogen, was used to control cooling. The sample was taken down to −50 °C to ensure freezing of the inclusions since the fluid in the inclusions shows pronounced supercooling effects. Most inclusions froze at ~−30 °C. The sample was allowed to warm up to a temperature 1–3 °C below the freezing point of the inclusions and was then usually refrozen to −21 °C. This promoted growth of observable single crystals of ice and often produced a displacement of the vapour bubble from its equilibrium position. The temperature of ice disappearance was then measured in large inclusions (~50–100 μm). In smaller inclusions ice was not in general visible[15] because of the small refractive index difference between ice and water in dilute solutions. The temperature at which the vapour bubble returned to its original shape and position was then recorded. The freezing points of the inclusions were close to 0 °C, so the apparatus could be precisely calibrated against the freezing point of deionised water. This was done by squirting a small quantity of vapour from deionised water over a sample at low temperature, to produce small crystals of ice immediately above fluid inclusions. The freezing points of the inclusions and the frost on the sample surface were then measured sequentially. Hence, the freezing points of the fluid inclusions and the deionised water were determined in identical experimental conditions. The result of 12 such measurements indicated that the recorded fluid inclusion freezing points had to be increased by 0.2 °C. The estimated reproducibility of any given fluid inclusion freezing point is ±0.2 °C for the particular temperature range encountered.

When fluid inclusions which were formed at temperatures greater than ~50 °C cool down naturally vapour bubbles nucleate and grow, because the coefficient of thermal contraction of the liquid is greater than that of the enclosing solid. The temperature at which the vapour bubble disappears during controlled heating may then be determined in order to obtain an homogenisation temperature, which, if pressure corrected when necessary[16,17], gives a temperature of formation of the fluid inclusion. Such homogenisation temperatures are readily measured since the small vapour bubble goes into a state of progressively more violent Brownian motion just before its disappearance. The apparatus was calibrated using substances of known melting point[14,15]. The accuracy of any given measurement was controlled by the observed range of temperature over which the standards melted, and is considered to be ±2 °C for the temperature range encountered.

Fluid inclusion freezing points

As the primary salinity of the hydrothermal hot water was the subject of interest, it was necessary to avoid samples which could have contained inclusions formed from a boiling fluid. Hence, material was examined from three relatively deep stockworks, in which pressure would have been highest. At Limni mine (Fig. 2) the main ore deposit occurs as a stockwork developed in pillow lavas[13,18]. Samples were examined from the core of the deposit, where copper grades of 1–3% occur. In the SE corner of the opencast pit pyritised and hydrothermally metamorphosed Sheeted Dyke Complex occurs as part of an upfaulted block. This locality is particularly valuable because it probably constitutes the best exposure of the deepest part of a stockwork anywhere in Cyprus. The metamorphic mineral paragenesis differs from that typical of higher level stockworks (quartz–chlorite–illite–sulphides[10]) and is characteristic of the greenschist facies (quartz–albite–chlorite–epidote–actinolite–sphene–pyrite). The third locality chosen was Alestos (Fig. 2) which consists of a small cupriferous pyrite stockwork developed in the zone transitional between sheeted dykes and pillow lavas (the 'Basal Group'). Three samples from each of these localities were studied.

Fluid inclusions were examined in vein quartz intimately intergrown with sulphides, which contains high densities of inclusions. The inclusions are of variable shape, some being very irregular in outline whereas others show well defined negative crystal morphologies. They are in general between 5 and 100 μm in size (Fig. 3*a*). Boiling during ore formation is usually indicated by the presence of two co-existing types of inclusion—one type, with a high vapour–liquid ratio, corresponding to 'steam' and the other, with a lower vapour–liquid ratio, corresponding to 'liquid'. The former homogenise in the gas phase, the latter in the liquid phase. Such evidence for boiling was searched for in these samples and not found. Most inclusions are of the simple two phase type, but a third solid phase was quite frequently present in larger inclusions. These daughter crystals occur as highly birefringent feathery aggregates similar in appearance to dawsonite ($NaAl\,CO_3\,(OH)_2$)[19] (Fig. 3*a*). The high birefringence suggests that they are some type of carbonate or sulphate.

The freezing points and homogenisation temperatures, most of which have been determined on the same inclusions, are given in Table 1 and all the freezing point data are presented as a frequency polygon in Fig. 4.

The inclusions are clearly of hydrothermal origin since their homogenisation temperatures are universally high. The data for the nine samples vary from 301±23 °C (21 measurements) to 351±4 °C (20 measurements). Most inclusions show no obvious geometrical relationships which can be used to indicate that they are primary (formed during growth of a crystal) or secondary (formed after growth). It could be argued, therefore, that they might be unrepresentative of the fluid which actually precipitated the ore minerals. This is shown not to be the case from examination of sample CY/75/39 from the main Limni

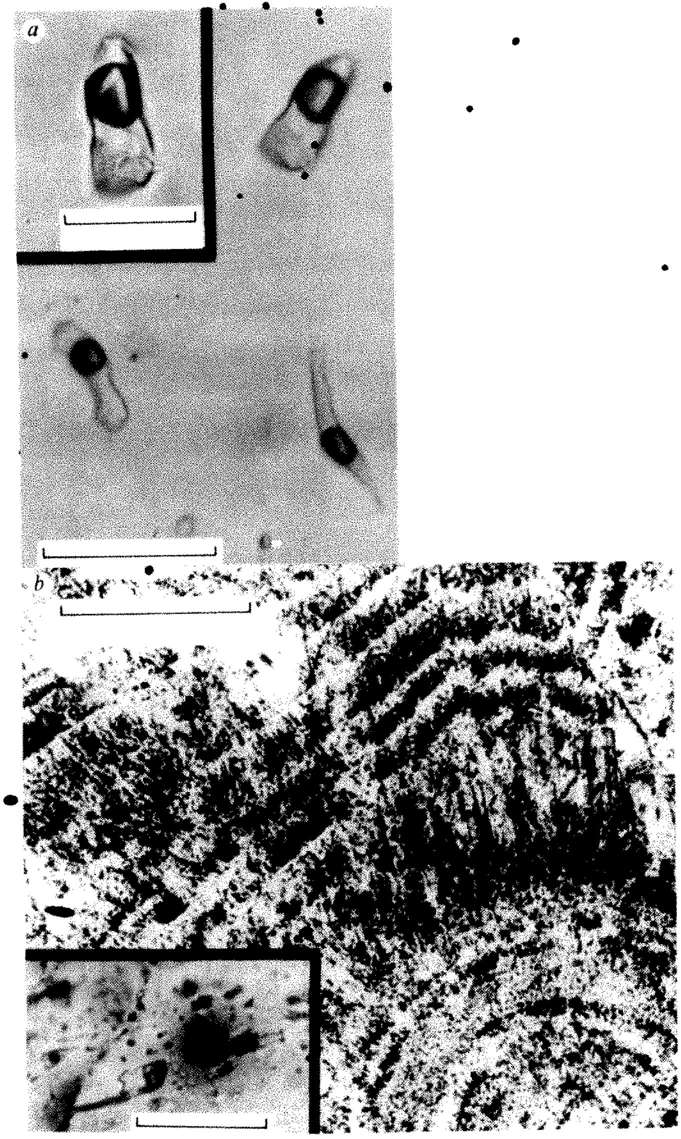


Fig. 3 *a*, Typical fluid inclusions in quartz from Cyprus sulphide ore deposit material (sample CY/75/38; Limni main stockwork; scale bar 30 μm). Central upper inclusion; T_H = 360 °C, T_F = −2.7 °C. Lower left inclusion; T_H = 313 °C, T_F = 2.1 °C. Lower right inclusion; T_H = 361 °C, T_F = −2.1 °C. Inset shows daughter crystals (? carbonate or sulphate) in the central upper inclusion, scale bar 20 μm. *b*, Primary fluid inclusions in vein quartz from the Limni main stockwork (sample CY/75/39). The inclusions define growth zones in the quartz crystal and are elongate parallel to the direction of growth. Therefore, according to normal geometrical criteria[16,17], they are considered to have formed during crystal precipitation. Since the vein quartz is intimately intergrown with sulphides the fluids in these inclusions are samples of the sulphide ore forming solution; scale bar 200 μm. Inset shows two primary inclusions in detail; scale bar 35 μm. Left inclusion; T_H = 307 °C, T_F = −2.0 °C. Right inclusion; T_H = 309 °C, T_F = −2.0 °C.

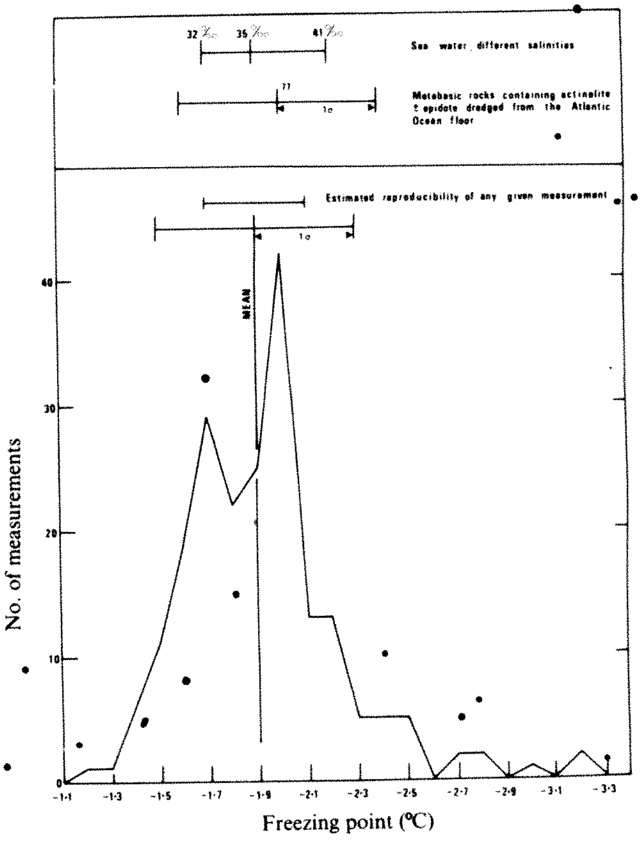
stockwork. This was the only sample to contain well defined primary inclusions clearly related to growth zones in quartz (see Fig. 3*b*). The results of measurements on these primaries and on inclusions of the more general type agree within 1σ (Table 1). Since the vein quartz is intimately intergrown with sulphides the fluid inclusion data from this sample, and all other samples, apply to the process of sulphide ore deposition itself.

The depressions of the freezing point of the fluid inclusions relative to pure water were found to be small (−1.6±0.2 °C to −2.4±0.4 °C), and therefore indicate that the hydrothermal ore forming fluid was a relatively dilute solution (2.7–4.0 equivalent weight % NaCl). The data for different samples are all statistically indistinguishable within 2σ limits, and most are indistinguishable within 1σ limits. Taking into account an accuracy of ±0.2 °C for any given measurement, the frequency polygon for all the 205 determinations (Fig. 4) shows a unimodal and symmetrical distribution about a mean value of −1.9 °C. The standard deviation is ±0.4 °C.

This freezing point is statistically indistinguishable from that of sea water. The salinity of sea water in the open ocean varies from 32 to 37.5‰ (ref. 20). In small ocean basins such as the Mediterranean and the Red Sea it rises to 41‰ (ref. 20). This produces a freezing point range for seawater of from −1.7 °C to −2.2 °C. Average seawater with a salinity of 35‰ has a freezing point of −1.9 °C. Hence, it is clear that a hydrothermal fluid identical to seawater in its salinity formed the ophiolitic sulphide ore deposits of Cyprus. That this fluid was actually of seawater origin is indicated by the stable isotope work of Heaton and Sheppard[4] and confirmed by the strontium isotope ratios reported by Chapman and Spooner[21], which demonstrate ^{87}Sr enrichment of mineralised material up to Upper Cretaceous seawater ^{87}Sr/^{86}Sr values. The hypothesis of metal leaching and transport by hot seawater is, therefore, verified.

The data may be compared with freezing points of fluid inclusions in minerals from greenschist facies metabasic rocks dredged from the Atlantic Ocean floor[15]. Since the mean homogenisation temperatures of six samples containing secondary actinolite ± epidote range from 219 °C to 324 °C, the fluids in the inclusions are clearly hydrothermal in origin. The mean of 77 freezing point values given by Jehl[15] for these samples is −2.0 °C, and the

Fig. 4 Frequency polygon of the 205 freezing point measurements of fluid inclusions in vein quartz from ophiolitic sulphide ore deposits, Cyprus, compared with the freezing points of seawater[20] and of fluid inclusions in metabasic rocks containing actinolite ± epidote dredged from the Atlantic Ocean floor[15]. Mean freezing point −1.9 °C, s.d. ±0.4 °C.



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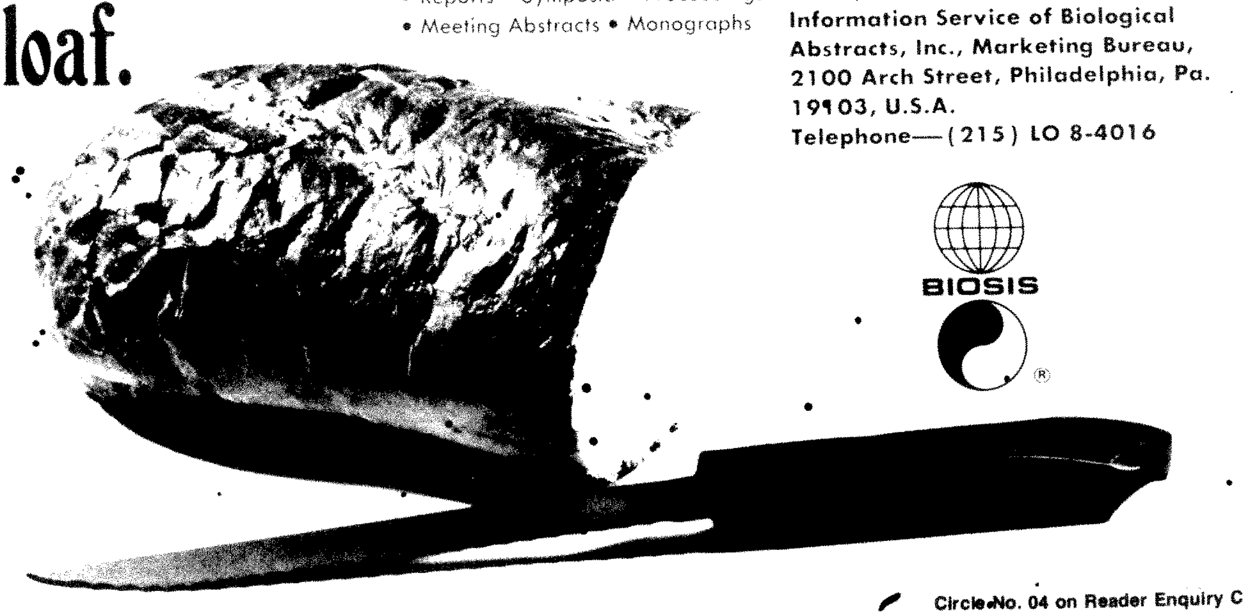

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Table 1 Freezing points and homogenisation temperatures of fluid inclusions in quartz from the Limni and Alestos ophiolitic sulphide ore deposits and comparative data

Sample No.	Sample locality and description	Freezing point (°C)	Homogenisation temperature (°C)
CY/75/38	Limni, main deposit. Quartz–pyrite–chalcopyrite–haematite–chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	−2.4±0.4 (20)*	309±26 (23)*
CY/75/39	Limni, main deposit. Quartz–pyrite–chalcopyrite–haematite–chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	−1.7±0.2 (20)	301±14 (30)
CY/75/39 (Primary inclusions)	Limni, main deposit. Quartz–pyrite–chalcopyrite–haematite–chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	−1.9±0.2 (21)	307±7 (21)
CY/75/52	Limni, main deposit. Quartz–pyrite–chalcopyrite–haematite–chlorite vein in hydrothermally mineralised and metamorphosed pillow lava	−1.6±0.2 (22)	301±23 (21)
CY/75/67A	Limni; pyritised and hydrothermally metamorphosed Sheeted Dyke Complex in the SE corner of the opencast pit. Quartz–epidote–chlorite–actinolite–calcite–pyrite vein	−2.1±0.4 (22)	309±19 (21)
CY/75/67B	Limni; pyritised and hydrothermally metamorphosed Sheeted Dyke Complex in the SE corner of the opencast pit. Quartz–epidote–chlorite–actinolite–calcite–pyrite vein	−2.0±0.2 (19)	309±22 (35)
CY/75/67C	Limni; pyritised and hydrothermally metamorphosed Sheeted Dyke Complex in the SE corner of the opencast pit. Quartz–epidote–chlorite–actinolite–calcite–pyrite vein	−2.0±0.2 (20)	322±10 (24)
CY/75/98A	Alestos. Quartz–pyrite–chalcopyrite–haematite–chlorite vein in hydrothermally mineralised and metamorphosed 'Basal Group'	−2.1±0.5 (21)	351±4 (20)
CY/75/98C	Alestos. Quartz–pyrite–chalcopyrite–haematite–chlorite vein in hydrothermally mineralised and metamorphosed 'Basal Group'	−1.9±0.3 (20)	314±7 (21)
CY/75/98D	Alestos. Quartz–pyrite–chalcopyrite–haematite–chlorite vein in hydrothermally mineralised and metamorphosed 'Basal Group'	−1.6±0.2 (20)	325±26 (20)
Mean value of all freezing points		−1.9±0.4 (205)	
Comparative data	Metabasic rocks containing actinolite ± epidote dredged from the ocean floor[15]	−2.0±0.4 (77)†	219–324†
	Kuroko sulphide ore deposits of Japan[23]	−1.8 to −3.7‡	146–189‡
	Sea water[20]	−1.7 to −2.2§	
	Average sea water (salinity = 35‰)	−1.9	

*Mean ± s.d.; figures in parentheses are numbers of measurements.

†These data were obtained from six samples (GA 12-14, V27-DR. 11-K1, G12-19, D1-11, G.12-A and AT150-20-1) listed by Jehl[15]. The number of measurements refers to the number of values quoted for each sample. The temperature range refers to the range of the means of the homogenisation temperatures listed for each sample. A wider range of salinities was recorded, but from material not from the greenschist facies[15,41].

‡This freezing point range was obtained from about 560 measurements on 51 samples from the Yunosawa deposit, Furutobe mine[23]. Fluid inclusions were studied in barite, sphalerite and fluorite. 34 samples were from siliceous ore and 17 from black ore. The homogenisation temperature range given refers to the range of the means of 85 determinations on seven samples (five from siliceous ore and two from black ore) also from the Yunosawa deposit[23].

§This range was obtained from the salinity variation in the open ocean (32–37.5‰) and from the salinities found in small ocean basins such as the Mediterranean (37–40‰) and the Red Sea (40–41‰)[20].

standard deviation is ±0.4 °C. This value is also statistically indistinguishable from that of seawater. These samples would probably have come from the recharge parts of ocean-floor hydrothermal convection systems, whereas the samples from the Cyprus ore deposits were formed in discharge zones. The data show, therefore, that seawater does not detectably change bulk composition sufficiently to affect its freezing point during interaction with basic igneous rocks at elevated temperature.

The freezing points of the Cyprus ore deposit fluid inclusions may also be compared with data from the Kuroko polymetallic sulphide deposits of Japan. These are also of submarine origin but formed in association with rhyolite domes rather than basic igneous rocks. Stable isotope ratios (δD and $\delta^{18}O$) of fluids from inclusions in sulphides in these deposits suggest that the ore fluid was also predominantly of seawater origin[22]. The range of ~560 measurements of freezing points of fluid inclusions in barite, sphalerite and fluorite from the Yunosawa deposit, Furutobe mine has been reported[23] as −1.8 °C to −3.7 °C (34 samples were from siliceous ore and 17 from black ore). This is very similar to the total range of fluid inclusion freezing points from the Cyprus ore deposits (−1.2 °C to −3.2 °C; Fig. 4). Hence, the effects on the bulk composition of seawater during water–rock interaction in the Cyprus and Kuroko environments were essentially the same.

Economic potential of oceanic crust layer 2

This information on fluid inclusion salinities in Cyprus ore deposits shows that when ordinary seawater interacts at elevated temperature with ordinary basaltic rock, sulphide deposits can be formed. Hence, regardless of whether the ophiolitic sequence of the Troodos Complex, Cyprus, is or is not a representative tectonically emplaced fragment of oceanic crust and upper mantle[24–26], the ore deposits within it may probably be taken as characteristic of the type of ore deposit formed during seawater circulation[1,27–30] within spreading oceanic ridges. This convective process is now thought to occur on a massive scale, and is probably the main hydrothermal ore forming process which affects the upper part of the sequence of igneous rocks of the oceanic crust (layer 2).

Hence, the sizes and metal contents of the Cyprus deposits may be used to assess the possible economic significance of oceanic crust layer 2. As mentioned above, these deposits are essentially small (0.5–15 million tonnes), occasionally relative rich copper ore bodies (~0.5–~4% Cu), which contain no other metals of economic importance[6-13]. To place them in context, they may be compared with a class of hydrothermal ore deposit which supplies the largest contribution (~45%) to world copper production—the porphyry copper deposits[31]. The average size of porphyry coppers is approximately 150 million tonnes, and they normally contain greater than 0.5% copper (average = ~0.8% Cu) which is often associated with economically significant quantities of molybdenum or gold[32]. They are therefore on average 1–2 orders of magnitude larger than Cyprus-type deposits, and can be much bigger. For example, the largest worked porphyry copper deposit (Bougainville, Territory of Papua and New Guinea) contains 900 million tonnes ore grading 0.48% Cu and 0.6 p.p.m. Au (ref. 33). The largest known porphyry copper (Cerro Colorado, Panama) contains at least 2,000 million tonnes ore grading 0.61% Cu (ref. 34). These data show, therefore, that Cyprus-type deposits are not particularly economically significant even when exposed on land. Also, an additional source of supply of copper in the future will probably be obtainable from the 'manganese nodules' which occur on the ocean floor[35,36].

Hence, bearing in mind the technological difficulties of ocean floor mining, this brief assessment of the ophiolitic sulphide ore deposits of Cyprus suggests that it is unlikely that layer 2 of the oceanic crust contains economically significant mineral deposits.

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Subsurface movements of stone artefacts and their implications for the prehistory of Central Africa

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The general and systematic character, in Central Africa, of a postsedimentary process of vertical redistribution of stone artefacts buried in a Kalahari sand mantle is strongly suggested. This invalidates the prehistoric nomenclature and chronology of this area and may have implications on the palaeoclimatological interpretation of stone-line profiles.

THE immense area occupied by the Zaïre or Congo river basin in the heart of Africa is still very little known from the archaeological point of view. The present-day nomenclature of the post-Acheulian prehistoric industries of Central Africa is based on very few excavations and on some systematic surface collections, essentially made in the regions of Kinshasa, in Zaïre[1] and of Lunda, in north-eastern Angola[2]. This nomenclature presents a somewhat conjectural and theoretical character as it consists only of a compilation of various observations made at different sites. Only at the site of the Gombe Point (formerly Kalina Point) at Kinshasa, which had been excavated[3-4] as early as 1925, was a complete post-Acheulian succession supposed to exist.

In 1968, a Symposium held at Tervuren, Belgium, under the auspices of the Commission of Nomenclature and Terminology of the Pan-African Congress on Prehistory and Quaternary Studies expressed the wish that new excavations should be undertaken at Gombe Point to determine and date this prehistoric succession[5]. These new excavations[6] were performed in 1973 and 1974. Fairly quickly, the conclusion was reached that this new research raised a series of problems much more important than the mere revision of the prehistoric nomenclature as it now seems that all the evidence, previously considered to be established, should be questioned.

Evidence from the Gombe excavations

At Gombe, the stone artefacts are distributed irregularly at all depths but with a considerable concentration (45%–95% of the total population) near the base of a homogeneous sand–silt–clay mantle, 3–5 m thick, which overlies a silicified bed-rock. This mantle is derived from the Red Sands of the Upper Kalahari, which cover the surrounding

Table 1 Correlation of the radiocarbon dates obtained at Gombe Point and in the regions of Kinshasa and Lunda

Industries	Gombe	Kinshasa	Lunda
-UPPER	GrN 7,219 : 3,385±35 b.p. GrN 7,278 : 5,260±40 b.p.	Lv 288 : 3,870±90 b.p. Lv 162 : 5,750±110 b.p. Lv 45 : 6,030±190 b.p.	UCLA 171 : 4,700±100 b.p. UCLA 167 : 6,830±120 b.p.
TSHITOLIAN		Lv 289 : 6,280±130 b.p.	
-LOWER		Lv 165 : 8,920±160 b.p. Lv 164 : 9,730±200 b.p.	UCLA 172 : 12,970±250 b.p.
LUPEMBO/TSHITOLIAN OR UPPER LUPEMBAN	GrN 7,220 : 8,095±50 b.p. GrN 7,276 : 14,840±80 b.p.	Lv 287 : 12,230±250 b.p. Lv 166 : 15,080±480 b.p.	C 580 : 11,189±490 b.p.* C 581 : 14,503±560 b.p.
SANGOAN/ LOWER LUPEMBAN	GrN 7,221 : 27,240±280 b.p. GrN 7,277 : >43,800 b.p.	Lv 163 : >26,000 b.p. Lv 44 : >30,000 b.p.	UCLA 169 : >34,000 b.p. UCLA 168 : 38,000±2,500 b.p. UCLA 709 : >40,000 b.p.

*This sample is associated with a material identified as Lower Tshitolian or Lupembo-Tshitolian.

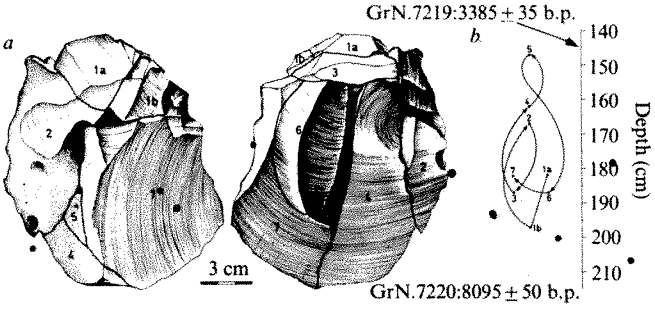
highlands[7]. This kind of irregular distribution of artefacts and this type of sediment are typical for the great majority of the open air archaeological sites known in the southern part of the Zaïre river basin and on which the knowledge of the Prehistory of Central Africa relies[8].

A study of the typological and quantitative distribution of the artefacts according to depth seemed to suggest the existence of different prehistoric industries: in the different trenches, various assemblages of characteristic artefacts were found in more or less the same order from the top to the base of the sand mantle. Charcoal fragments found at the same levels revealed dates which fell within the same periods as those already obtained for analogous industries from Kinshasa and Lunda. A fair typological and chronological correlation could thus be noted between the industries of the southern Zaïre river basin (Table 1). It must, however, be emphasised that at Gombe, just as at Kinshasa and Lunda, the dated samples exhibited no other relation with the archaeological material than to have been found at the same depth as some characteristic artefacts.

However, a systematic study of the reassembly of the worked stones of Gombe (that is, joining together the tools, flakes, core and fragments struck off from the same block) has shown that fitting artefacts were found at several different depths from which different radiocarbon dates have been obtained (the vertical distance between joining pieces sometimes exceeds 1 m). Hundreds of analogous observations have been made through the whole thickness of the sand mantle.

Fitting artefacts are part of the same technological process (blades removed from a blade core for instance) and are strictly contemporaneous. In most cases, the vertical distribution of the elements of the reconstructed cores does not follow the order in which they have been struck off (Fig. 1). This excludes the hypothesis of a later reutilisation of older artefacts. The abundance of reassembled pieces also precludes important lateral transport. Finally we were led to the hypothesis that only subsurface movements of the worked stones could explain this important vertical dispersion of contemporaneous artefacts. On the evidence of the reassembly, it becomes clear that, contrary to appearances, the site of Gombe Point has been disturbed in some way and that the lithic assemblages discovered there at a given depth are not homogeneous. As the Gombe Point can be considered as one of the most complete and most representative prehistoric sites of the southern Zaïre basin, it was thought important to investigate more deeply these disturbances.

Fig. 1 *a* Reassembled blade core from Gombe Point and *b*, vertical distribution of its elements. Numbers indicate the order in which the flakes have been removed: 1a and 1b are fragments of the first flake, 7 is the core itself.



Experimental research

By means of experiments[9], the possibility of subsurface movements[10–11] and of a dispersion of artefacts was tested out. The original materials were used for the experiments. The texture and some mechanical properties of the sediment are given in Table 2.

We investigated whether artefacts, laid down on and/or buried in the sediment could undergo vertical movements, when the sediment is wetted and dried alternatively. Down to depths of 60 cm sand columns were used. Greater depths were simulated by putting down weights on the sediment column. The different methods of wetting and drying were

Table 2 Characteristics of the sediment used in the experiments

Sand content (> 63 μm)[2]	58.1%	
Silt content (2–63 μm)	28.0%	
Clay content (< 2 μm)	13.9%	
Median size	77 μm	
Modal size	95 μm	
Calibrage	very bad	
Liquid limit water content	23%	
Plastic limit water content	12%	
Plasticity index	11%	
Specific weight	2.63 g cm^{-3}	
Angle of internal friction	45°	water content: < 1%
Cohesion	0 g cm^{-2}	
Apparent angle of internal friction	15°	water content: between 20% and 23%
Apparent cohesion	50 g cm^{-2}	
Compressibility constant:		
dry	25.9	slow test
wet (19% water content)	25.6	
Permeability: 10^{-3} cm h^{-1} for a dry volume weight of 1.42 g cm^{-3}		

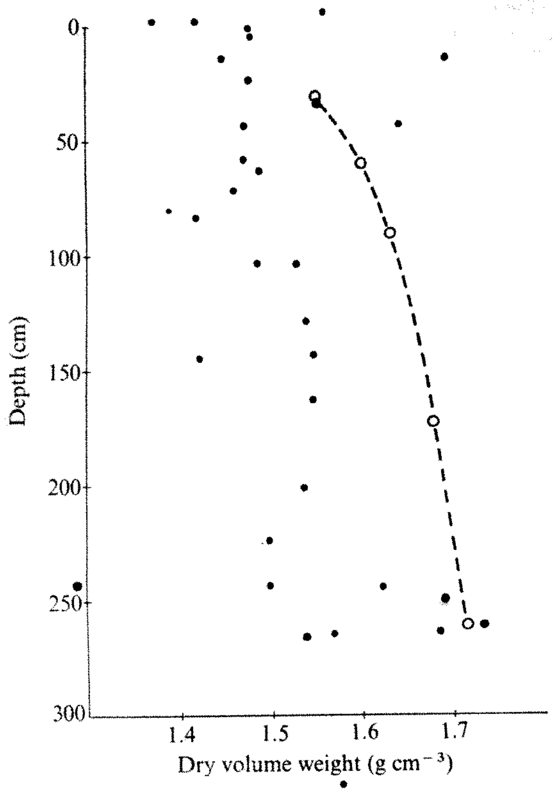


Fig. 2 The dry volume weight of undisturbed soil samples taken from trench 2 at Gombe (●) and the dry volume weight derived from the experiments and the oedometer-test (dotted line). In establishing the latter, a supposed dry volume weight of 1.5 g cm^{-3} of the column above each point was accepted. Therefore the dotted line still gives underestimated values.

simulations of an oscillating water table or of the percolation of rain water from the surface. Three conclusions were reached:

(1) Artefacts laid down on the surface can penetrate into the sediment over a distance of some mm to more than 1 cm, depending on their weight and shape. The amount of penetration shows a linear relation with the logarithm of the number of wetting–drying cycles applied. The explanation lies in a slight compression of the sediment just beneath the artefact. The experiment showed that the weight of the artefacts becomes unimportant and negligible, once they are covered by some cm of sediment.

(2) Buried at greater depths, artefacts do not move vertically, except if the artificial soil column is not entirely consolidated. Artefacts, laid down in an unconsolidated sediment at real or simulated depths of 30, 60, 90 and more than 200 cm, displayed, during consolidation, a general tendency to accompany the descent of the level at which they were buried. Some of them did however descend considerably more or less than the sediment which originally surrounded them.

(3) The dry volume weight of the sediment, measured in the basal part of the different experimental boxes after each experiment, exceeds systematically that of undisturbed samples, taken in the field at depths obtained or simulated in the laboratory (Fig. 2). An oedometer test confirmed the dry volume weight values obtained from the experiments.

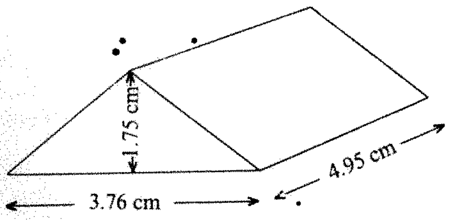
The observation in the field of termite activity and the fact that undisturbed soil samples displayed in several cases a high density of very small (ø about 0.5 mm) biogenic galleries led to the conclusion that the sediment mantle at Gombe is still unconsolidated. It undergoes a double post-sedimentary process: the soil fauna brings up particles to the surface. The dry volume weight of the sediment will diminish along collapsing biogenic structures. A continuous biogenic action and subsequent collapse of the structures

Table 3 Movement indices as measured in the shearing box of a shearing apparatus

Block number	Sediment	Orientation of block	Number of attempts	Mean movement index
1	wet	point up	6	1.16±0.12
	wet	point down	10	0.89±0.21
	dry	point up	5	1.51±0.21
	dry	point down	5	1.28±0.21
2	wet	point up	5	0.95±0.14
	wet	point down	5	0.97±0.15
	dry	point up	5	1.25±0.16
	dry	point down	5	1.38±0.17
3	wet	point up	5	1.18±0.05
	wet	point down	5	0.98±0.08
	dry	point up	5	1.37±0.24
	dry	point down	5	1.28±0.20

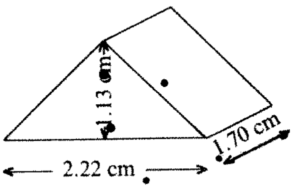
The movement index $i = (\Delta h_0)/(\Delta h_s)$, where Δh_0 is the absolute amount of descent of the gravity centre of the object during exertion of vertical stress on the soil column; Δh_s is the absolute decrease in height of the part of the soil column situated before the measurement between the bottom of the box and gravity centre of the object. For $i < 1$ the object penetrates in sediment situated originally above. In relative terms, the object rises. For $i > 1$ the object penetrates in sediments situated originally below. In relative terms, the object descends.

Block 1



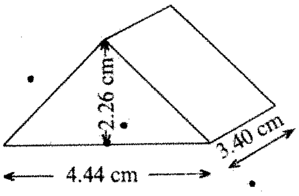
Centre of gravity 1.24 cm below top

Block 2



Centre of gravity 0.80 cm below top

Block 3



Centre of gravity 1.60 cm below top

created in this way, must finally lead to a reconsolidation of the soil column. In fact, the sediment mantle at Gombe must be considered as consolidating permanently in reaction to the continuous biogenic upworking.

In this perspective, conclusion (2) (see above) becomes very significant and was therefore elaborated on by further experiments, simulating the consolidation of the sands by applying vertical stresses. Measurements were made in the shearing box of a monoaxial shearing apparatus: it was noted on several occasions that undeformable objects did not, under compression, strictly accompany the vertical movement of the enclosing sediment. Depending on dimension, form and orientation of the objects and on the water content of the sediment, the objects penetrated into sediments originally situated above or below them. Table 3 gives the vertical movement indices for three wooden blocks.

It should be pointed out that the variability in shapes and dimensions of a lithic industry is obviously considerably higher than that of three wooden blocks. Therefore, the range of vertical movement indices within a whole industry will be also much wider than for the three blocks. Moreover, the experiments are based on the theoretical conception of a homogeneously spread biogenic activity. This is not the real situation in nature. Preferential reworking can contribute to a much higher dispersion than expected on the base of the experiments. Finally, these experiments yield information only about dispersion during consolidation. It is clear that the biogenic activity itself can also create vertical displacement of artefacts.

Discussion

At Gombe, the reassembly shows that strictly contemporaneous artefacts are scattered irregularly over the whole thickness of the homogeneous sand mantle. The experiments confirm the possibility of a postsedimentary differential descent of artefacts in the soil column. This process is catalysed by biogenic activity.

The following facts strongly suggest that this process of redistribution has a general and systematic character in the entire area covered by Kalahari-type sands in Central Africa:

(1) The sites on which our present knowledge of the Prehistory of Central Africa relies are located in the same environmental background—the southern Zaïre river basin.

(2) In these sites, the artefacts are generally distributed in a similar way—"isolated and at no consistent level"[8]—within a homogeneous mantle of redistributed Kalahari sands.

(3) At Lunda, Kinshasa and Gombe, typologically identical assemblages of worked stones are dated within exactly the same periods.

(4) Although systematic reassembly of lithic material has only been carried out for Gombe, similar examples of joining pieces found at different depths have also been observed at Dinga (Kwango) and at Kamoa (Shaba)[12], thus proving that Gombe is not an isolated case.

The most evident result of this redistribution process is a mixing up of originally superposed occupation layers and in most cases the inability to decide if one or more industries are present. The post-Acheulian industries that have been defined in Central Africa probably represent nothing more than heterogeneous assemblages of stone artefacts, without any stratigraphical, typological, chronological or ethnographical significance. The radiocarbon dates give only the age of various charcoal and wood samples but have no direct relation with the archaeological material.

The redistribution process also presents an interest for Quaternary stratigraphical interpretations in the area considered here. Part of the basal concentration of artefacts at Gombe cannot be interpreted as a residual pavement. The contemporaneity of several pieces of this concentration and of some artefacts scattered in the overlying sand mantle can only be explained by a differential descending velocity: artefacts with higher movement indices have already got down to the bed-rock, while others are still descending. The distribution of coarse elements in many stone-line profiles in Central Africa resembles that of the artefacts at Gombe. Many of these stone-lines could perhaps be considered as the result of descent through the overlying mantle[10-11], instead of a residuum, concentrated during an erosion phase. This has implications for the palaeoclimatological interpretation.

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Defective interfering particles with covalently linked [±]RNA induce interferon

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Defective interfering (DI) particles of vesicular stomatitis virus which contain covalently linked complementary [+]message and [−]anti-message RNA as a single-stranded ribonucleoprotein complex within the particle, are extremely efficient inducers of interferon. A single particle can induce a quantum yield of interferon. A single molecule of double-stranded RNA presumed to form, at least in part, on entry into the cell is thought to induce interferon synthesis. Conventional [−]RNA DI particles with the same polypeptide composition as [±]RNA DI particles fail to induce interferon.

LAZZARINI *et al.*[1] have described a defective interfering (DI) particle of vesicular stomatitis virus (VSV), DI-011, which contains covalently linked complementary [+] message and [−] anti-message (genomic) RNA. The [±] RNA exists within the particle as a single strand of 8×10^5 daltons in a ribonucleoprotein complex 0.85 μm long, and on deproteinisation 80–90% of the RNA self-anneals into a double-stranded (hairpin) structure 0.33 μm long[1].

The availability of pure populations of these defective interfering particles which contain RNA capable of assuming a double-stranded configuration and which can specifically bind to and enter cells, provides a unique system for delivering a

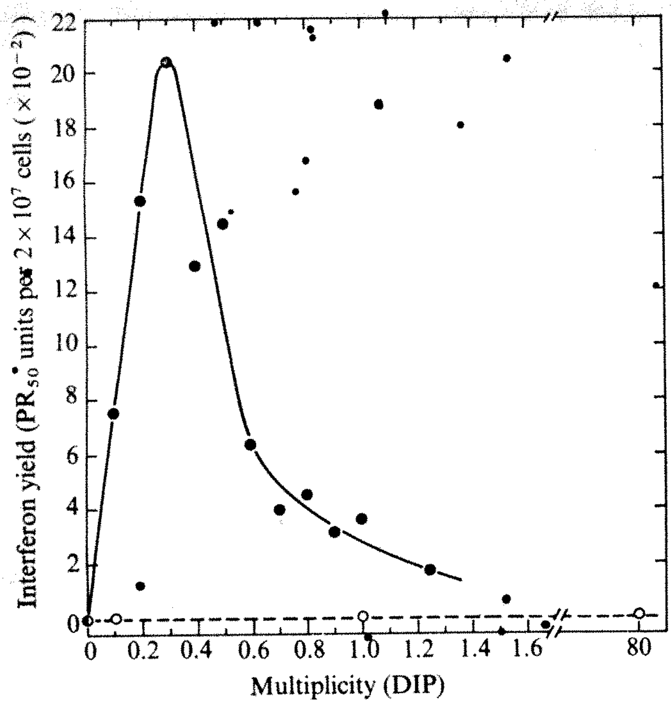


Fig. 1 Interferon inducing capacity of [±]RNA DI-011 (●) and [−]RNA DI-HR (○) particles. Primary chick embryo cells aged *in vitro* for 6 d (ref. 6) were infected with varying multiplicities of DI particles obtained as thrice gradient-purified stocks. DEAE-dextran at 10 μg ml^{-1} was present during virus attachment. Following virus attachment at 37.5 °C for 30 min cell monolayers were washed three times with warm medium and incubated at 40.5 °C with 3 ml of attachment solution[28] per 60-mm dish. After 24 h the supernatant fluid was collected and assayed for interferon as described previously[15]. Activity was recorded as a 50% VSV plaque reduction unit (PR$_{50}$ unit ml^{-1})[15]. Interferon yield is reported as the total amount of interferon released into the medium from a monolayer of 2×10^7 cells in 24 h. This period sufficed to produce maximal yields of interferon over the entire range of multiplicities tested. DI particle titres were determined by measuring the reduction in yield of thrice gradient-purified homologous VSV-HR B particles[8,9].

single molecule of [±] stranded RNA to the cell in conditions which preclude RNA replication[2]. We have used this system to determine whether the [±] RNA of DI-011 particles might assume in the cell a configuration of biological significance beyond that of its role as a DI particle. We examined the function of DI-011 as a defective cell-killing particle[3,4] in conditions where conventional [−] strand RNA particles do not kill cells[3], and tested their capacity to induce interferon production[5,6]. We present evidence here that DI-011 particles do not kill cells, even at multiplicities of infection up to 50, but that a single particle, presumably because of its potential to form double-stranded (ds) RNA and the capacity of dsRNAs to induce interferon[5,7], suffices to induce a quantum yield of interferon, making it the most efficient inducer of interferon discovered so far.

Interferon induction by DI-011

Primary chick embryo cell monolayers aged *in vitro* for 6 d (ref. 6) were challenged with various multiplicities of DI-011 particles (m_{DIP}), on the basis of interfering particle activity[8,9]. Our basic observation is illustrated in Fig. 1 as a dose (m_{DIP})–response (interferon yield) curve and demonstrates that preparations of DI-011 particles contain highly efficient interferon inducers. These data, typical of seven experiments, reveal that initially, increasing the multiplicity of DI-011 particles produced a sharp increase in the yield of interferon, with peak titres (at 24 h) obtained at about $m_{DIP} = 0.3$. (This value ranged from $m_{DIP} = 0.1$ to 1 in seven experiments.) Further increases in m_{DIP} resulted in a marked reduction in interferon yield. The magnitude of the changes in interferon yield observed on increasing the dose of DI-011 particles in the low multiplicity range seemed characteristic of dose–response curves where one particle elicits a quantal response but two or more do not. Consequently, we compared experimentally derived dose–response curves with those expected from a Poissonian distribution of DI particles for cases where quantal yields of interferon would derive from classes (subpopulations) of cells attaching r particles at different multiplicities, m, and values of r (ref. 10). In Fig. 2 we compare the expected curve from the Poisson distribution for the class of cells infected by only one particle ($r = 1$) with the observed data from two experiments. The multiplicity of DI-011 particles which produced the maximum yield of interferon was determined by the least squares method for a best fit curve, with the logarithm of the interferon yield used to minimise the percentage error in the assays for high and low values of interferon. Curves representing other values of r ($r = 2$, $r > 1$, $r > 2$) are not shown, but revealed marked deviation from the observed results. From the goodness of fit of the observed dose–response curve to that expected from the class of cells infected with only one particle of DI-011 ($r = 1$), we conclude that infection with a single particle of DI-011 suffices to induce a quantum yield of interferon—in effect, defining an 'interferon-inducing (IF) particle'. An equivalency between interferon-inducing particles and physical particles is implicit in Lazzarini's observation that

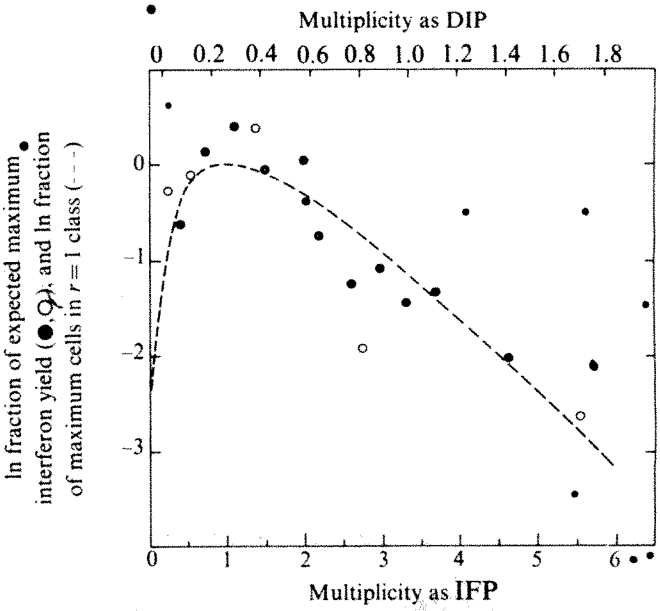


Fig. 2 Comparison of the observed dose (multiplicity)–response (interferon yield) data with those expected if only cells which received one particle were full yielders of interferon—representing the $r = 1$ class of the Poisson distribution where

$$P_{(r)} = (e^{-m}\,m^r)/r!$$

In this case, m is the multiplicity of DI-011 particles, and $P_{(r)}$ the fraction of cells infected by r particles of DI-011. The class of cells which is infected by a single particle (that is, the case where $r = 1$) is $P_{(1)} = me^{-m}$ (ref. 10). ●, ○, Data from Fig. 1 and one additional experiment. - - - -, Curve derived from the $r = 1$ class of the Poisson distribution. The upper abscissa shows the multiplicity of infection based on the DI particle titre whereas the lower abscissa defines the DI-011 stock in terms of the number of interferon-inducing (IF) particles. On the ordinate, the 'expected' maximum interferon yield was based on a maximum derived from a least squares fit of the experimental points. For these experiments, the ratio IFP:DIP ≃ 4, and indicates that more virions can function as interferon inducers than as DI particles. In this regard, Lazzarini consistently finds at least a threefold excess of physical particles, as assayed by electron microscopy, to DI particles, as measured by the interference test[8,9] (personal communication). Allowing for this factor of three, the multiplicity on the top and bottom abscissae become almost the same, indicating an equivalency of physical and IF particles.

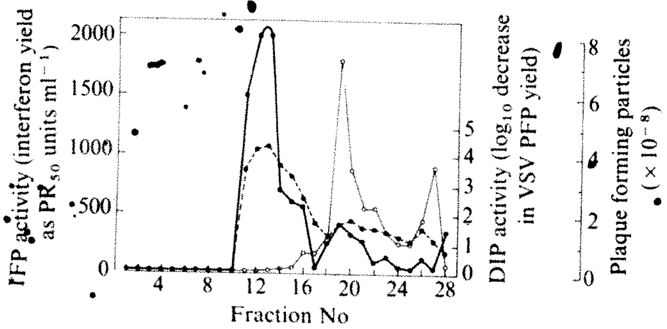


Fig. 3 VSV-MS(DI-011) profiles of plaque-forming particle (○—○), defective-interfering particle (● - - - ●) and interferon-inducing particle (●—●) activity following velocity sedimentation on a linear sucrose gradient. A partially purified preparation of VSV-MS(DI-011) labelled with ^{3}H-uridine and containing roughly equivalent amounts of B and T particles (kindly supplied by R. A. Lazzarini) was applied to a 17 ml 5–40% linear sucrose gradient and centrifuged at 75,000*g* for 120 min at 4 °C. Fractions were collected from the top of the gradient and analysed for TCA-precipitable radioactivity (data not shown), plaque-forming particle, defective-interfering particle and interferon-inducing particle activity as described elsewhere[3]. Assays of interferon inducing activity for each fraction were carried out at optimal multiplicities for interferon induction (Fig. 1).

there is about a threefold excess of physical particles to defective-interfering particles in purified preparations of DI-011 (R. A. Lazzarini, personal communication). The data also indicate that cells which attach two or more DI-011 particles yield little or no interferon. Factors affecting the production (synthesis and release) of interferon and an antiviral state in this multiplicity range will be reported elsewhere.

Conventional [—] RNA particles representing a common one-third length component from the Indiana HR strain of VSV did not induce interferon when tested similarly (Fig. 1).

Comparison of different types of particle

A stock containing DI-011 and standard infectious B particles was obtained from BHK-21 cells in conditions which produced roughly equivalent amounts of DI and B particles. These particles were concentrated by centrifugation, and profiles of interferon-inducing, defective-interfering and plaque-forming particle activity established by testing each fraction of the gradient for these respective activities as described previously[3] (Fig. 3). These results demonstrate that the interferon-inducing and defective-interfering particle activity profiles are essentially coincident throughout the gradient, with both activities peaking at fractions 12 and 13. Infectivity in the form of plaque-forming particles peaks at fraction 19. We conclude that the interferon-inducing particle activity of DI-011 particle preparations corresponds in sedimentation characteristics to those of defective-interfering particles.

Prevention of interferon induction

Inhibitors of cellular RNA and protein synthesis, such as actinomycin D and cycloheximide respectively, prevent interferon induction[5]. Table 1 demonstrates that when either of these drugs is present together with an optimal multiplicity of DI-011 interferon-inducing particles, little or no interferon is produced. Thus, it seems that new cellular mRNA and protein synthesis are required for the production of interferon which follows infection with DI-011 particles.

When preparations of DI-011 particles are exposed to antiserum specific for the Indiana serotype of VSV, there is a marked reduction in the capacity of that preparation to induce interferon (Table 1). We conclude that the molecule(s) responsible for interferon induction in populations of DI-011 particles gain entry into the cell through particles which can be specifically neutralised with VSV antiserum.

Effects of heat or ultraviolet radiation

Stocks of gradient-purified DI-011 particles were exposed to different doses of ultraviolet or heated for 20 min at 50 °C and tested for their capacity to induce interferon. Table 1 shows that heated DI-011 particles remained fully functional as interferon inducers. The same heat treatment when applied to complete virions reduced infectivity, cell-killing particle activity, and virion-derived transcription 100-fold[4]. Figure 4 shows that the interferon-inducing capacity of DI-011 particles is extremely resistant to inactivation by ultraviolet. A dose of ultraviolet (2,615 erg mm^{-2}) equivalent to 50 and 10 lethal hits to plaque-forming and cell-killing particle activity, respectively[4], had virtually no effect on interferon induction by DI-011 particles, but reduced the interfering capacity of DI-011 particles 10-fold or more (data not shown).

Induction of antiviral state in different cell types

Some cell systems seem to acquire an antiviral state on stimulation with interferon inducers even though little or no interferon may be detected[11,12]. Consequently, we tested for intracellular viral interference in cells in which the yield of interferon induced by DI-011 particles was abundant (primary chick embryo cells)[3], low (human FS-4 cells)[13] or nil (GMK-Vero cells)[14]. Monolayers of these three cell types were challenged with various multiplicities of DI-011 particles. After incubation for 20 h, the supernatant fluid was removed and assayed for its interferon content[15]. To establish the degree of intracellular viral interference, the cell monolayers were challenged with Sindbis virus ($m_{PFP} = 5$) in the presence of actinomycin D (1 μg ml^{-1}). The data in Table 2 reveal that DI-011 particles induce an heterologous antiviral state capable of reducing the yield of challenge Sindbis virus in chick embryo cells by 1,000-fold or more—even at the multiplicities of inducer particles which produce relatively low yields of interferon ($m_{IFP} > 2$). However, we note that the yield of interferon at these multiplicities, although low compared with the titres obtained at $m_{IFP} = 1$, is nonetheless significant ($\simeq$ 100 units ml^{-1}) and could itself account for development of an antiviral state during the 20-h test period. This consideration does not hold for human FS-4 cells, since little or no interferon was detected over the 100-fold range of multiplicities tested and illustrated in Table 2. Thus, DI-011 particles in human FS-4 cells produced an antiviral state in the apparent absence of released interferon. The heterologous antiviral effect (challenge with Sindbis virus) which develops in these human cells on infection with DI-011 particles indicates that the yield of interferon from cells is but one measure of DI-011 particle

Table 1 Factors affecting the induction of interferon by ±RNA DI-011 particles

Treatment of [±]RNA DI-011	Interferon yield (PR_{50} units per 2×10^7 cells)
Untreated (control)	37,000
Actinomycin D (1 μg ml^{-1})	25
Cycloheximide (50 μg ml^{-1})	< 20
VSV antiserum*	< 20
Heat (50 °C for 20 min)	33,000
Ultraviolet†	39,000

In all experiments an optimal multiplicity of DI-011 particles, that is, $m_{IFP} = 1$, was used to achieve maximal interferon yield as measured 24 h after infection. Actinomycin D or cycloheximide were added to the medium immediately after DI particle attachment and remained throughout the 24-h test period. The antiserum used during virus attachment could reduce the plaque-forming particle titre of a VSV Ind. stock by 99.9% in 30 min. The heat treatment reduced cell-killing, plaque-forming (and virion-transcription) activity each by 99% (ref. 4).

*Treatment with normal rabbit serum at a comparable dilution produced control (untreated) values of interferon.

†Figure 4 illustrates the exact relationship between ultraviolet dose and interferon induction by DI-011 particles, and compares it with the surviving activity for infectivity (plaque formation).

Table 2 Induction of intracellular interference against Sindbis virus by [±]RNA DI-011 particles in different cell types

Multiplicity (DI-011)	Sindbis virus yield (PFP ml^{-1}) Primary chick embryo cells	Human FS-4 cells	GMK-Vero cells
0	6.2×10^9	6.4×10^9	9.4×10^9
0.1	1.9×10^6	1.2×10^{10}	4.1×10^9
1	4.9×10^5	3.4×10^9	1.2×10^{10}
10	4.4×10^6	1.2×10^8	1.2×10^{10}
100	9.0×10^5	3.6×10^6	4.8×10^9

Monolayers of cells were infected with varying multiplicities of once gradient-purified [±]RNA DI-011 particles. Virus attachment was carried out for 30 min at 37.5 °C in attachment solution[28] containing 10 µg ml^{-1} DEAE–dextran. Monolayers were washed three times with warm medium and incubated at 40.5 °C (chick cells) or 37.5 °C (FS-4 or GMK-Vero cells) with 3 ml of medium per 60-mm dish. After 24 h, supernatant fluids were collected, processed, and assayed for interferon as described previously[15]. These monolayers were washed twice with attachment solution and infected with wild-type Sindbis virus at m_{PFP} = 5–10. Actinomycin D (1 µg ml^{-1} for chick cells and 10 µg ml^{-1} for FS-4 and GMK-Vero cells) was included during virus attachment to prevent any further induction of interferon[6]. Cells were washed four times to remove unattached virus and 3 ml of medium was added to each plate. Infected monolayers were incubated at 37.5 °C for 10 h and the supernatant fluid titred by plaque assay for the Sindbis virus yield.

action on the cell; another is development of an intracellular antiviral state[11].

However, we have not ruled out the possibility that very small amounts of interferon are being produced[12], nor have we determined whether the reduced efficiency of inducing an antiviral state reflects a lowered probability of attaining a double-stranded configuration of [±] DI-011 RNA in FS-4 cells. The results with GMK-Vero cells are clear—no interferon is produced, nor is an antiviral state achieved by DI-011 particles in this cell which seems genotypically incapable of responding to inducers of interferon[14].

Effect of [−]RNA defective interfering particles

Some standard preparations of DI particles might consist of both [−] and [±] (also [+]?) type particles, and although [−]DI particles do not induce interferon (Fig. 1), they might interfere with, or enhance, the interferon-inducing capacity of [±]RNA particles. We tested this possibility by simultaneously infecting primary chick embryo cells with a multiplicity of DI-011 optimal for interferon induction (m_{IFP} = 1) and a high multiplicity of a conventional [−]DI-HR particle (m_{DIP} = 50). These large numbers of [−]RNA DI particles reduced the interferon-inducing capacity of DI-011 more than 10-fold, even though this multiplicity of [−]DI-HR particles had no adverse effect on the cells as judged by examination under phase contrast optics, or by counting single cells by the window technique[16]—confirming earlier observations that these particles are not intrinsically toxic[3,8,9,17]. We are continuing to study this effect.

[±]RNA DI-011 particles are not intrinsically toxic

Because a single non-infectious particle of VSV—a defective cell-killing particle—suffices to kill a cell[3], it is important to use highly purified populations of DI particles to test for their intrinsic toxity[3,8,9,17]. Preparations of [±]RNA DI-011 particles gradient-purified three times did not affect the plating efficiency of single GMK-Vero or chick embryo cells as tested up to m_{DIP} = 50, and so behaved like conventional [−]RNA DI particles[3]. Multiplicities in excess of 50 [±]RNA DI particles resulted in a measurable decrease in plating efficiency and the appearance of a cytopathic effect in monolayer cultures. It is not clear whether these effects reflect a cumulative toxic effect of these particles or the presence of otherwise non-detectable levels of infectious cell-killing particles. We suspect the latter, since inclusion of VSV antiserum in the medium shortly after infection prevents the progressive cytopathic effect observed in monolayer cultures infected at m_{DIP} > 50.

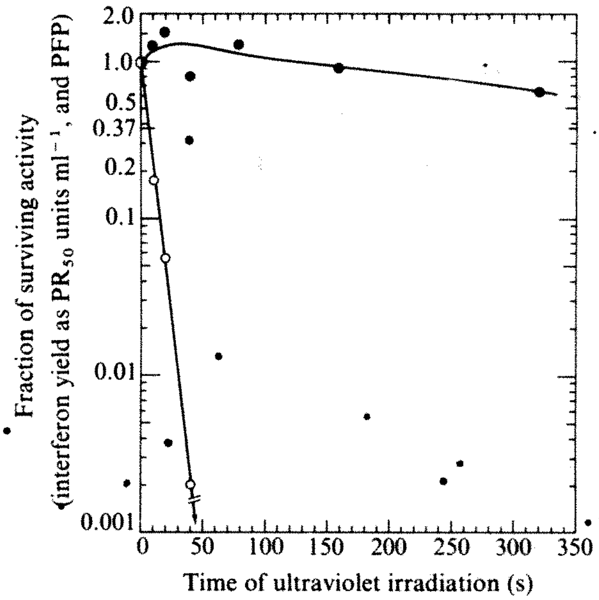
Single particle of [±]RNA DI-011 induces interferon

Our data indicate that a single [±]RNA DI-011 particle suffices to induce a quantum yield of interferon, and does not kill cells. The dose (multiplicity)–response (interferon yield) curve generated by DI-011 particles fits best a model where cells infected with a single particle (the $r = 1$ class of the Poisson distribution)[10] synthesise and release a full yield of interferon, thus identifying a new attribute of these subvirions—their capacity to act as discrete interferon-inducing (IF) particles. The dose–response curve also indicates that cells infected with two or more IF particles respond differently and yield little or no interferon.

Requirement for dsRNA

We infer that interferon induction results from the action of a helical structure formed from some, if not all, of the complementary bases which make up the covalently linked [+] message–[−]anti-message single-stranded RNA of the infecting DI-011 particle. In the same conditions, single DI-HR particles of conventional [−]RNA composition do not induce interferon. Thus, at an average of about 0.3 DI particle per cell, [±]DI-011 and [−]DI-HR induce the synthesis of approximately 25,000 and < 10 PR_{50} (VSV) units of interferon respectively per 2×10^7 aged chick embryo cells[6]. That a double-stranded RNA formed intracellularly is the putative inducer of interferon in cells infected with DI-011 can also be inferred from the fact that [±]DI-011 and [−]DI-HR particles both obtain polypeptides from viral transcripts synthesised by the coinfecting standard B particle of VSV[18]. R. Lazzarini has shown by double isotopic labelling and coelectrophoresis that the polypeptide composition of DI-011 is identical with that of the parental infectious type (personal communication). DI-011

Fig. 4 Interferon-inducing capacity of ultraviolet-irradiated DI-011 particles. Comparison with plaque-forming particle activity. One-half millilitre of thrice gradient-purified DI-011 particles and of wild-type VSV Ind. (strain MS) infectious B particles were added to separate 100-mm Petri dishes and exposed to a germicidal lamp (2,735 Å) which delivered 65.4 erg mm^{-2} per 10 s. Samples of irradiated particles were tested either for interferon-inducing capacity (IFP, ●) as in Fig. 1, or for plaque-forming activity (PFP, ○).



and DI-HR particles apparently share identical polypeptides therefore, differing only in their content of covalently linked [+]message–[−]anti-message, and [−]anti-message RNA, respectively. In addition, neutralisation of the interferon-inducing capacity of DI-011 preparations with specific antiserum to VSV (Indiana) demonstrates that the inducer molecule gains entry into the cell in a particle which possesses the specificity of the Indiana serotype, as do these DI-011 particles.

It will be difficult to prove directly the existence of a helical structure of [±]RNA at the level of the one molecule per cell presumed to form on interaction of a cell with a single particle of DI-011, particularly if once inside the cell, the number of base-pairs required for inducer function is as few as the six to twelve reported necessary for poly(rI)·poly(rC) to function similarly[19]. We do not know how the inducer molecule assumes a functional configuration in the cell, but it seems likely that in aged chick embryo cells (and perhaps to a lesser extent in human FS-4 cells) sufficient viral nucleoprotein is removed from the viral ribonucleoprotein core to permit a threshold amount of base pairing. Conceivably, only the [+] stranded moiety of the [±]RNA functions as inducer. The high yield of interferon produced on infection with only a single DI-011 particle, presumably one molecule of [±]RNA, makes it, on a molar basis, and in terms of a delivery system, the most efficient inducer of interferon so far discovered.

The fact that the interferon-inducing capacity of the DI-011 particle is not affected by heat and ultraviolet is consistent with the [±]RNA's being the molecule responsible for interferon induction[20]. We do not know yet whether a given molecule of [±]RNA from DI-011 can serve both as a template for replication, as in homotypic interference, and an inducer of interferon.

Possible role of [±]RNA particles in virus infection and disease

The remarkable efficiency of the [±]RNA DI-011 particle in inducing interferon and/or an antiviral state, coupled with the fact that interferon-mediated interference can prevent cell killing by VSV[15,21], suggests that the [±]RNA class of DI particle might play a part in persistent infection and viral disease beyond that presently postulated for its conventional [−]RNA counterpart[2]. Involvement of the interferon system in persistent infection would provide a mechanism whereby cells can survive in the presence of lethal virus—an aspect not satisfactorily accounted for by present models. Thus, although a single DI particle can prevent completely the replication of its standard infectious helper virus[3,8,9], it does not prevent cell killing by that coinfecting virus[3]. Furthermore, we have demonstrated that single cells infected with an otherwise lethal dose of VSV, survive and form normal-sized colonies if, before infection, they have acquired an interferon-mediated antiviral state[15]. Current experiments provide evidence of a role for [±]RNA DI particles in persistent infection. They demonstrate that pretreatment of mouse L cells with [±]RNA DI-011 particles renders them refractory to cell killing by wild-type VSV, a property not shown by [−]RNA DI-HR particles in the same conditions (M.J.S. and P.I.M., in preparation). We also note that the only DI particles of several types tested by Holland and Villarreal[17] which are found consistently to establish persistent infection in BHK cells coinfected with VSV, were those derived from stocks of *ts*-mutant G31(III). We have tested a preparation of long DI particles prepared from this mutant as supplied by E. Reichmann (obtained from J. Holland), and found that it acted as an excellent interferon inducer (data not shown). We also note that the presence, or generation of [±]RNA DI particles might account for the induction of interferon in mice by VSV[22], or rabies virus (refs 23, 24 and references contained therein). We are now examining different types of DI particle for their IF particle activity, and by implication, for the presence of [±]RNA containing particles.

Characterisation of a closely related [±]RNA DI particle of VSV corroborates the existence of this new class of defective interfering particle[25]. The ease with which single molecules of DI-011 [±]RNA can be delivered to the cell, and their efficiency at inducing interferon synthesis and/or an antiviral state makes them new and potent tools to define and understand the many effects of dsRNA on cells[7,26,27].

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Note added in proof: We have observed that the sparing of cells from the lethal action of VSV (and Newcastle disease or Sindbis virus) which develops upon pretreatment with DI–011 particles is obliterated completely when antiserum to interferon is present during the pretreatment. These results provide further evidence that the antiviral state induced by the DI–011 particle to heterologous viruses is interferon mediated.

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letters to nature

Q0000−398 is a high-redshift quasar with a large angular size

REDSHIFTS of between 2.5 and 3.1 have been measured for nine optically-selected quasars as part of the Cerro Tololo survey of the Southern Hemisphere (M. G. Smith, see ref. 1). None of these quasars were known to be radio sources. We chose several accessible quasars from Smith's list as a starting point in our investigation to see if they might not be radio sources. We report that Q0000−398 is a quasar of high redshift and large angular size.

During October, 1976, we detected two of the quasars using the three-element interferometer of the National Radio Astronomy Observatory (NRAO), Green Bank, West Virginia,

Table 1 Data on quasars of considerable angular extent

Source name	1950.0 Radio position h m s					Measured flux density S_{2695} (Jy)	Redshift z	Angular extent θ (arc s)
Q0000−398A	00 00 29.78	(.4)	−	39°49′ 27″.	(15)	0.013	2.827	134″
Q0000−398B	00 00 27.15	(.4)	−	39 47 13.	(20)	0.015		
OE323A	03 13 46.19	(.4)	+	34 26 27.8	(3.)	0.073	1.16	27.4
OE323B	03 13 44.38	(1.)	+	34 26 31.1	(3.)	0.135		
M0758+120*	07 58 14.41	(.1)	+	12 01 42.5	(.8)	0.08	2.66	see text
OS210A	16 06 10.74	(.1)	+	28 56 43.1	(.2)	0.10	1.99	31.1
OS210B	16 06 09.34	(.1)	+	28 57 06.1	(.2)	0.15		

*In Table 1 of Baldwin *et al.*[6] the optical and radio positions are reversed.

with a 300–1,200–1,500-m baseline configuration, operating at 2,695 MHz. The radio map for Q0000−398 (18 mag, z = 2.827) shows two weak components separated by 134±40 arc s. If these components are associated with the optical object, Q0000−398 is the quasar with the largest known angular size at its redshift value. (Inspection of the Whiteoak extension print of this area of sky shows no galaxies in this area, which may be emitting this radiation.)

The second quasar investigated, Q0049−393 (18 mag, z = 2.94) was also detected (S_{2695} = 26 mJy, where 1 mJy = 10^{-29} W m^{-2} Hz^{-1}), but was unresolved.

These two quasars were observed along with several other

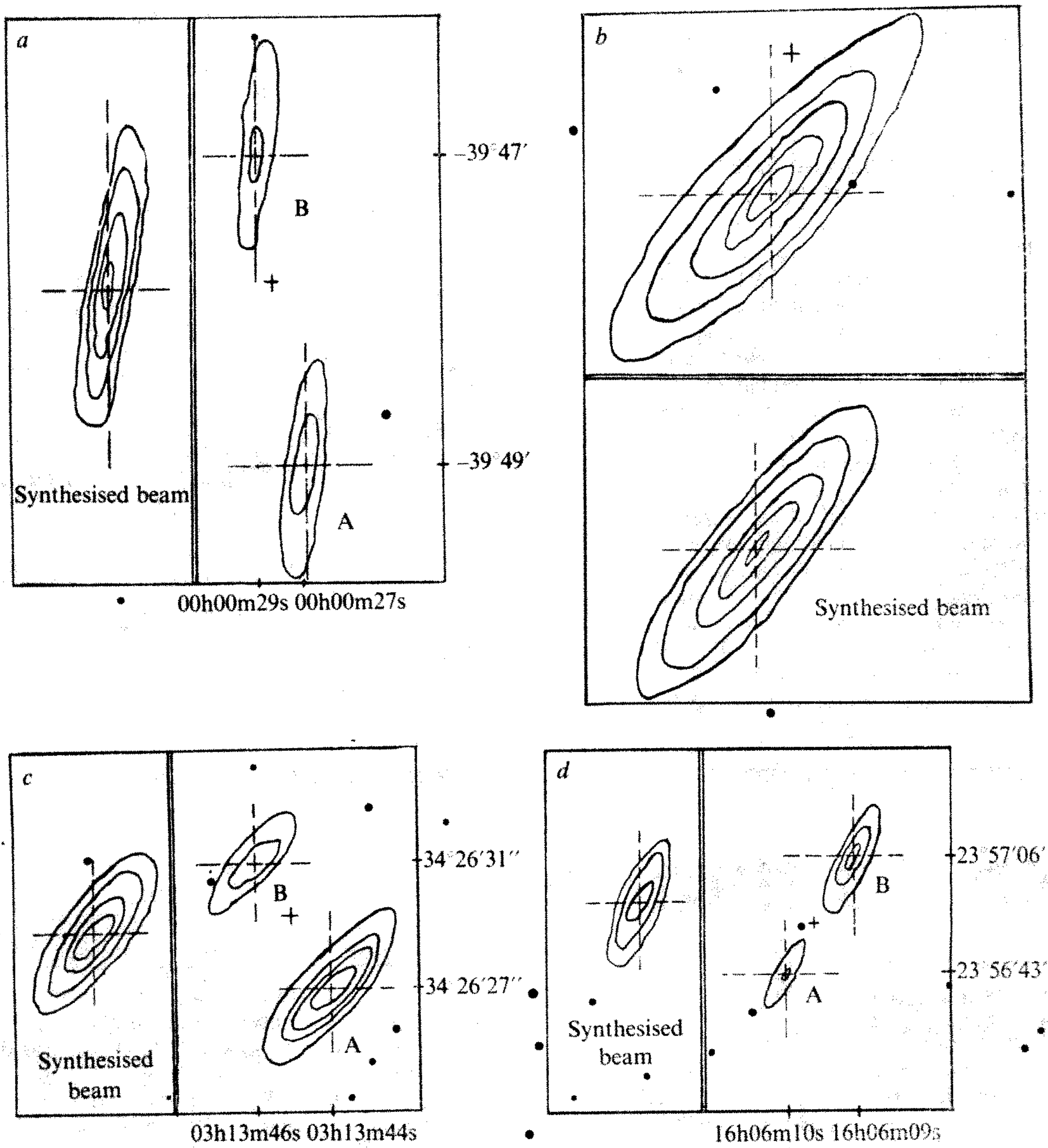

Fig. 1 Radio contour maps with synthesised beams at 2695 MHz for *a*, Q0000−398; *b*, M0758+120; *c*, OE323 and *d*, OS210. Crosses represent optical centroids. The angular separation of the radio and optical centroids for M0758+120 is about 14 arc s.

steep-spectrum quasars listed in Table 1. All of the sources were observed in ideal weather conditions with good hour angle coverage in the *u–v* plane (allowing for the constraints of the elevation angle limit at low declinations, roughly ±3h at −39°). Details regarding the operation and characteristics of the interferometer are given in ref. 2.

Results for the quasars found to have considerable angular extent are presented in Table 1. Figures 1 *a–d* present clean maps at 2,695 MHz of four of the sources studied: Q0000−398, OE323, M0758+120, and OS210. Synthesised beams accompany each map. The radio component associated with M0758+120 is shifted by about 14 arc s from the optical counterpart. Further studies are required to determine if a fainter, weaker radio component is present, or if the radio source is actually associated with the quasar.

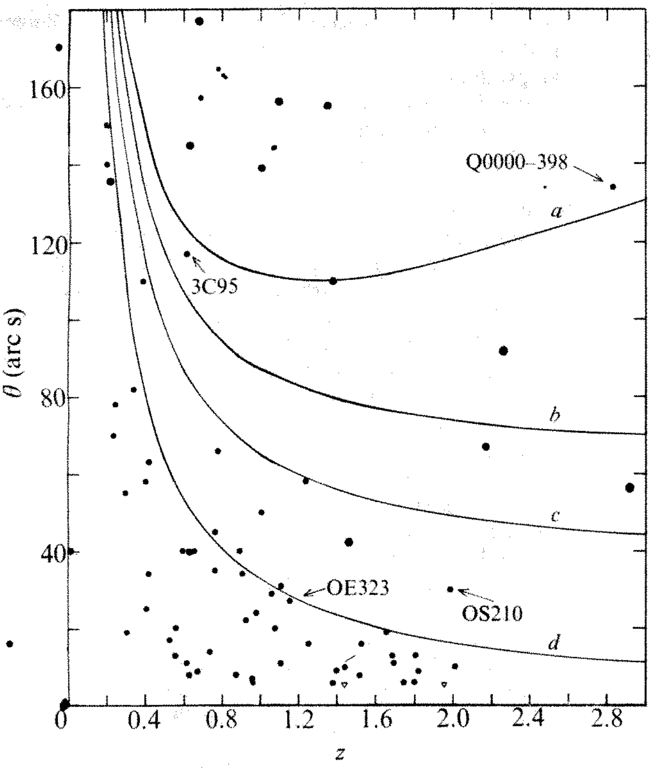

Fig. 2 Angular size–redshift diagram for 55 quasars with spectral indices $\alpha(408\text{–}1{,}415\,\mathrm{MHz}) \geq 0.80$ and $\alpha(1{,}415\text{–}5{,}000\,\mathrm{MHz}) > 0.60$. Curves *a–d* demonstrate the behaviour of a standard rod of 950 kpc in Einstein–de Sitter, Milne, de Sitter (steady state) and static Euclidean universes, respectively. Triangles represent upper limits. A Hubble constant of $H_0 = 50\,\mathrm{km\,s^{-1}\,Mpc^{-1}}$ is assumed.

Assuming that quasar redshifts are cosmological, Friedmann models predict that quasars with constant linear dimensions will exhibit a minimum angular size as a function of redshift. The Einstein–de Sitter model, for example, with deceleration parameter, $q_0 = 1/2$, predicts a minimum near $z = 1.2$. Wardle and Miley[3] have found that the maximum angular size of a sample of 166 quasars decreases with increasing redshift much faster than predicted by any conventional cosmological model. They conclude that evolutionary effects are responsible for the apparent Euclidean behaviour of the upper envelope to the angular size redshift diagram. The large angular extent of Q0000−398, however, tends to weaken this conclusion and suggests that a minimum may exist in the upper envelope function (see Fig. 2).

Additional evidence that the angular dimensions of quasars may exhibit a minimum is indicated by the results of Hewish *et al.*[4]. Their study of the angular sizes of compact components of strongly scintillating 3CR sources shows evidence of the mean angular sizes increasing with higher redshift, as one would expect in evolving cosmological models with q_0 in the range of 0.5 to 2.0. Readhead and Hewish[5] conclude that the apparent deficiency of small-diameter compact sources at large redshifts is real and significant.

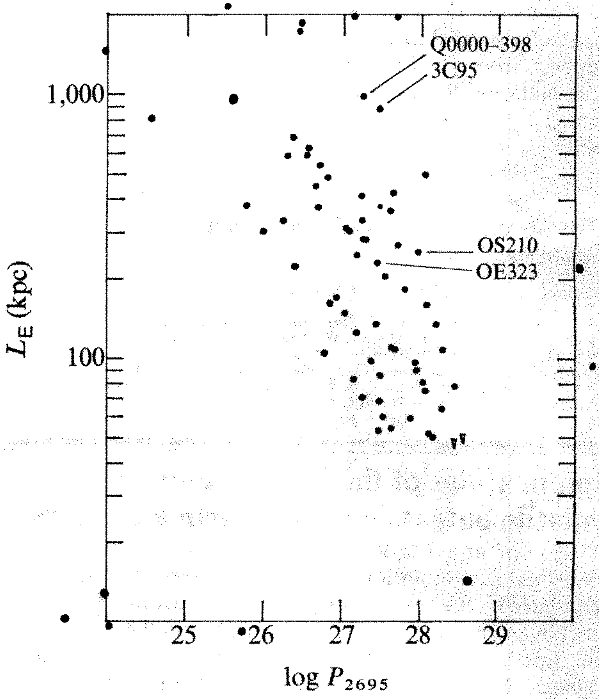

Fig. 3 Linear separation L_E (kpc) against 2,695 MHz spectral power (W Hz^{-1}) for Einstein–de Sitter model. Data are for the quasars in Fig. 2. Triangles represent upper limits.

Although Wardle and Miley[3] find no evidence for radio selection effects as a result of the sample being selected by flux density, since Q0000–398 was selected optically and has a much lower flux density than most of the quasars in their sample, this suggests that radio selection effects may be important. A radio selection effect can arise from samples being selected by flux density if the luminosity of quasars decreases with increasing separation of components.

The largest angular-size quasars tend to have much steeper spectra than found for small-diameter quasars. Using the spectral-index criteria that $\alpha(408\text{–}1{,}415\ \mathrm{MHz}) \geq 0.80$ and $\alpha(1{,}415\text{–}5{,}000\ \mathrm{MHz}) > 0.60$ (where $S \propto \nu^{-\alpha}$) eliminates most small-diameter quasars as well as those with low-frequency cutoffs from the Wardle–Miley diagram. The remaining subset of 52 quasars, along with the other new data presented in this paper, is shown in Fig. 2. Figure 3 shows the relation between radio luminosity at 2,695 MHz and linear size for the quasars in Fig. 2. An Einstein–de Sitter model and a Hubble constant of $50\,\mathrm{km\,s^{-1}\,Mpc^{-1}}$ has been assumed. The subset of 50 quasars shows an apparent inverse correlation (coefficient = −0.63, significant at the 0.01 level) between luminosity and linear size (2 quasars with upper limit angular sizes were not included). No correlation between luminosity and linear size has been found for quasars having either spectral index flatter than the criteria set above. These quasars are more likely to possess self-absorbed components, which may smear out any relation between luminosity and linear size.

The results reported here demonstrate the importance of considering radio selection effects in the angular diameter–redshift diagram. Since any radio selection effects are removed when quasars are selected optically, more extensive mapping programs should be undertaken, looking specifically for large-scale structure around optically-selected, high-*z*, quasars.

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Implications of the Viking results for volatile outgassing from Earth and Mars

TUREKIAN and Clark[1] have recently discussed an inhomogeneous accumulation model for terrestrial planets, have compared the outgassing product from a veneer of carbonaceous chondrites with the abundances of volatiles on Earth and predicted the amounts of various gases for Venus. The recent measurements of rare gas abundances on Mars by Viking[2–4] have prompted us to test such a model for Mars. We find that not only the relative abundance of H, C, N, ^{20}Ne, ^{36}A, Kr and Xe but their total amounts can be best explained if the outer layers of Earth and Mars were made of material similar to ordinary chondrites (LL) as suggested by Anders[5], rather than carbonaceous chondrites as assumed by a number of authors in recent years.

In a non-homogeneous accretion model[1,5,6] the planet is supposed to have accumulated from at least two different types of material. The accretion starts in a relatively hot solar nebula and therefore the inner part of the planet contains mostly high temperature condensates such as metallic iron, magnesium silicates, Ca–Al–Ti and rich oxides. As the planet grows, it is heated by the release of gravitational energy and if it is large enough (compare Earth and Venus) the proto planet becomes completely molten and liquid iron and nickel sink to the centre. With the passage of time both the proto planet and the solar nebula cool and the growing planet can then acquire a layer of low temperature condensates containing relatively large amounts of volatiles such as carbon, water, nitrogen and the rare gases. If the upper layers could then get heated again either by the radioactive decay of ^{40}K, ^{238}U and ^{232}Th or by some other mechanism, the volatiles contained in the low temperature condensates can be released to the surface of the planet. The suggestion is that this process was responsible for the formation of the atmosphere and oceans of the Earth.

Table 1 Volatiles on Earth, CCl and ordinary chondrites (g cm^2) and atom ratios normalised to carbon

	H	C	N	^{20}Ne	^{36}A	^{84}Kr	^{132}Xe
Atmosphere, hydrosphere, crust, upper mantle (*a*)*	35,800	52,800	1955	1.3×10^{-2}	4.1×10^{-2}	2.0×10^{-3}	1.2×10^{-3}
Carbonaceous chondrites (Cl) (*b*)†	34,300	(52,800)	4990	3.5×10^{-4}	2×10^{-3}	5.9×10^{-5}	7.6×10^{-5}
Ordinary chondrites (LL) (*c*)‡	24,200	(52,800)	3010	5.3×10^{-3}	3.6×10^{-2}	5.2×10^{-4}	6.4×10^{-4}
Atom ratios (*a*)/(*b*)	1.0	(1)	0.4	37	20	33	15
Atom ratios (*a*)/(*c*)	0.7	(1)	0.7	2.1	1.1	3.8	1.8

Hydrogen content data from LL ordinary chondrites taken as 0.05% by weight (Geiss, personal communication).
*From ref. 1.
†From van Schmus and Hayes and Mazor *et al.* quoted in ref. 1.
‡Data from ref. 15.

Accepting this model in principle, we wish to discuss the nature of condensates which can most reasonably explain the volatile inventory on the surface of Earth and Mars.

Table 1 shows the total abundances of six volatile elements present in the Earth's atmosphere, hydrosphere, crust and upper mantle, adopted from Turekian and Clark[1]. The second line shows the abundances of these elements if they represent outgassing from carbonaceous chondrites (also from Turekian and Clark). Here we normalise the abundances for carbon. (Assuming 3.5% concentration by weight in the carbonaceous chondrites, complete outgassing from a 4-km layer of CCl would correspond to the abundance of carbon on Earth). Line 3 which represents the ratio of Earth to CCl shows that the agreement is far from satisfactory for the case of rare gases. Disparities of up to a factor of 37 exists for the case of ^{20}Ne.

A similar treatment using the volatile content of ordinary chondrites (LL) rather than CCl, produces a more satisfactory result. Line 4 is the total abundances of volatiles outgassed from a layer of ordinary chondrites, again normalised for carbon. Because of relatively low abundance of carbon (0.12%) in ordinary chondrites, line 4 represents outgassing products from a layer 116 km thick. The ratios shown in line 5 indicate a much better overall agreement for ordinary chondrites than for CCl (line 3).

Table 2 shows a similar treatment for Mars. Here of course there are more unknowns than on Earth, especially for C, N and H which may be in the surface layers rather than the atmosphere. We therefore follow a slightly different approach in constructing the table. Line 1 shows the abundances of rare gases, carbon and nitrogen measured in the atmosphere. Line 2 shows the amounts which would be expected if the outgassing is from the ordinary chondrite type material and normalised for ^{36}A. Line 3 then shows the amount of volatiles which may be in the surface layers of Mars or have escaped the planet.

The agreement for Kr and Xe is reasonably good. This leads us to presume that such an approach to the deduction of the total outgassing on Mars may be valid. If so, a number of inferences can be drawn from Table 2 as follows.

(1) The total volatile inventory on Mars corresponds to complete outgassing from an 0.5-km layer of ordinary chondrites or, if the layer is ~100 km thick (compare Earth), the outgassing has been only 0.5% effective.

(2) 145 g cm^{-2} of C corresponds to ~500 g cm^{-2} of CO_2. As only a very small amount of CO_2 is in the atmosphere, relatively large amounts (>90%) may be in the regolith, as proposed earlier by Huguenin[7].

(3) 8 g cm^{-2} of nitrogen either lies buried in the surface or has escaped the planet[8].

(4) 66 g cm^{-2} of H corresponds to ~600 g cm^{-2} of H_2O. Although the hydrogen content of the ordinary chondrite is the most poorly known piece of data in Table 2, it is interesting to note that the recent Viking results provide two interesting pieces of data which are relevant in this case. First, the surface layers of Mars seem to contain ~1% H_2O (ref. 9). If we assume that the regolith is ~100 m thick this would correspond to ~200 g cm^{-2} of H_2O in the Martian surface. The second Viking observation relevant here is that the residual polar cap in the nothern hemisphere is made of water ice[10]. Although there are large uncertainties in the estimates of ice thickness in the polar cap, our inventory of water outgassing on Mars would

Table 2 Martian volatile inventory derived by degassing from ordinary chondrites (g cm^{-2})

	H	C	N	^{20}Ne	^{36}A	^{84}Kr	^{132}Xe
Mars atmosphere observed	—	5.0	0.50	$< 10^{-4}$	1.0×10^{-4}	$\sim 6 \times 10^{-6}$	$\sim 1 \times 10^{-6}$
Ordinary chondrite (LL)	66	145	8.3	10^{-5}	1.0×10^{-4}	2×10^{-6}	$\sim 3 \times 10^{-6}$
Volatiles escaped or on surface	66	140	7.8	–	–	–	–

allow a thickness of up to 40 m, if escape of water from Mars can be ignored. If not[11], the polar cap thickness would be accordingly smaller.

(5) Major volatiles deduced from Table 2, H_2O, CO_2, N_2, add up to ~1,100 g cm^{-2}. It has been suggested[12] that Mars goes through major climatic fluctuations and at times all volatiles could be in the atmosphere. According to our inventory the total mass of the atmosphere in such a case would be ~1,100 g cm^{-2} which, for Martian gravity, corresponds to ~400 mbar; an atmosphere composed of ~50% CO_2 and ~50% H_2O with traces of nitrogen.

The ^{40}A degassing problem is a very different question than that of general outgassing of the planet (such as CO_2, H_2O, rare gases and N_2). ^{40}A is a radiogenic product of ^{40}K and was nearly completely absent in the earlier stage of planetary formation (its degassing should be a function of its production and could be continuing at the present time (compare the Moon))[13].

Viking measurements showed an ^{40}A/^{36}A ratio in the Martian atmosphere 10 times greater than the ratio in the Earth's atmosphere[2]. Soil sample analyses[14] give an upper limit for the potassium concentration at the surface of 0.25% (compared with the potassium concentration of 1.6% (ref. 1) in the Earth's crust).

We calculated the necessary thickness of a layer to account for the observed quantity of ^{40}Ar in the Martian atmosphere assuming two different values for the potassium concentration: 0.25% which is upper limit from Viking experiments and 0.08% which is the concentration in ordinary chondrites (LL). We find that layers of 2.5 km and 8 km respectively can account for the results of Viking measurements. These thicknesses are to be compared to the half-kilometre thickness calculated by us previously to account for the other volatiles (Table 2). This difference implies a different mechanism for outgassing of ^{40}A compared with the rest of Martian atmosphere.

The fact that the absolute amount of ^{40}A in the atmosphere of Mars could be explained by ~10 times less K in the upper layers of Mars than on the Earth, is interesting. It implies that the upper layers of Mars have not been substantially enriched in K and other radioactive elements perhaps because of the absence of extensive melting in the interior of Mars during its formation.

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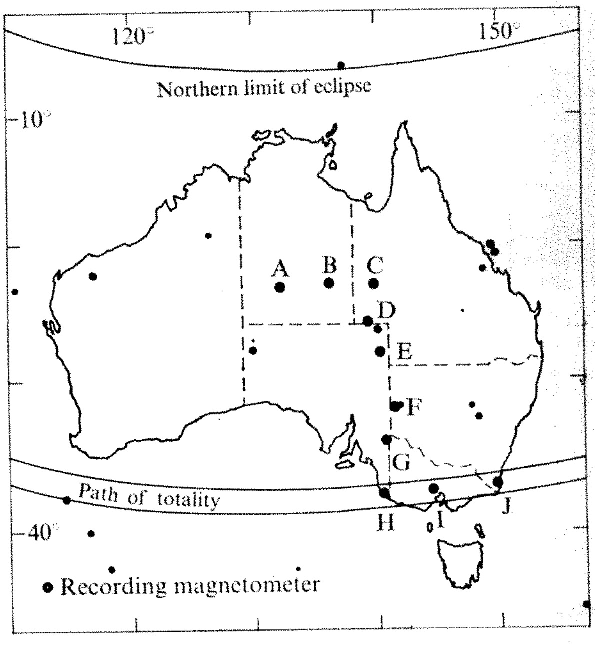
Magnetic observations of the solar eclipse of 23 October 1976 in Australia

SOLAR eclipses may cause geomagnetic effects by disturbing the flow of electric currents in the ionosphere[1,2], and a number of observations of this phenomenon have been made in recent years[3,4]. To monitor any magnetic effects of the eclipse of 23 October 1976, we operated recording magnetometers at 10 sites in central and eastern Australia (Fig. 1); these instruments were in addition to the regular observatories of the Bureau of Mineral Resources near Perth and Melbourne. We present here a preliminary analysis of the declination variations recorded in the path of totality at stations H (Millicent, S.A.) and J (Merimbula, N.S.W.).

The recording instruments were Gough-Reitzel variometers[5], set to take readings at intervals of 10 s. Because any eclipse effect was expected, on elementary grounds, to be mainly in the declination component, the declination sensors of the instruments were increased in sensitivity by a factor of four above their normal level. Timing of the 10-s readings (on film) was estimated to be accurate to within a few seconds.

The day of 23 October 1976 proved to be magnetically quiet in the declination component. Minor activity occurred at 0711 h Universal Time (UT), about 30 min after eclipse totality had passed. The declination records for stations H and J have been digitised at 10-s intervals for a 4-h period centred on the eclipse, normalised by calibration factors, and are shown plotted in Fig. 2.

Fig. 1 Sites of recording magnetometers operated to monitor the 23 October 1976 eclipse. Site H is Millicent Airfield (lat. 37°35′S, long. 140°22′E). Site J is Merimbula Airfield (lat. 36°55′S, long. 149°53′E).

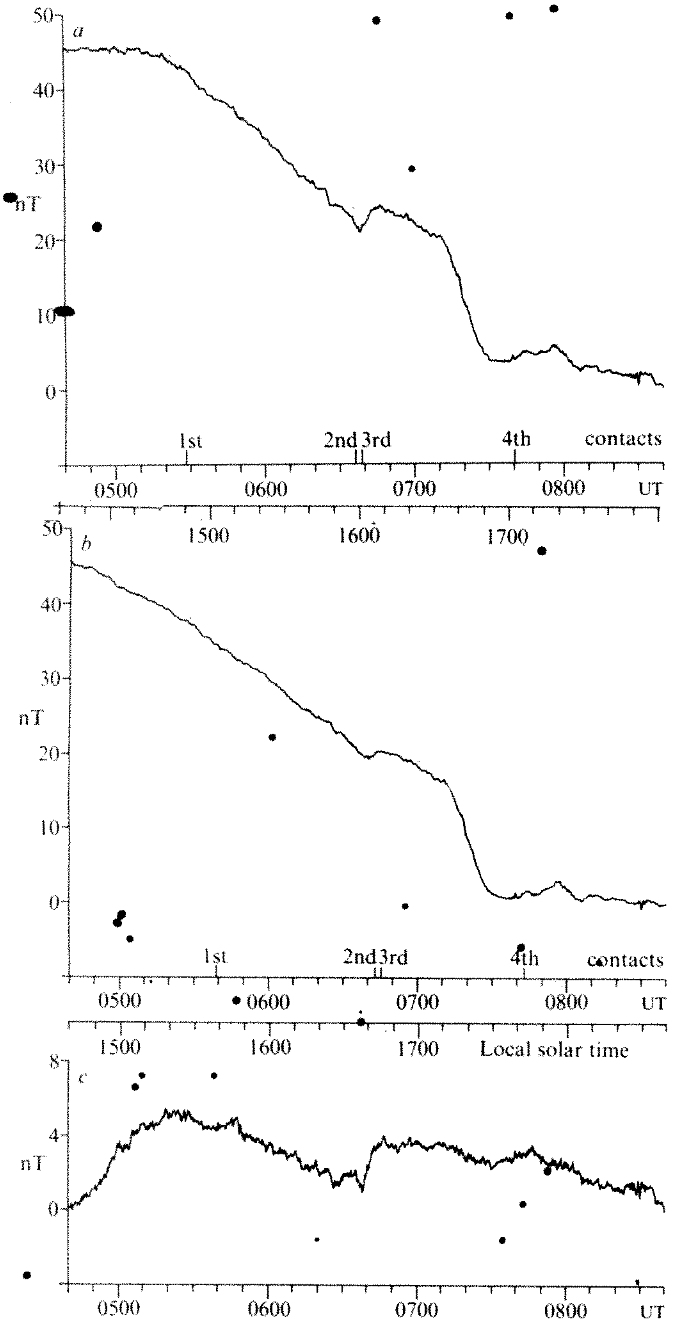


Also shown in Fig. 2 is the difference between them, subtracting trace J from trace H.

Natural electromagnetic induction in south-east Australia has been well studied by two previous magnetometer array experiments[6,7], which showed the coast effect to be strong in the vertical component of magnetic fluctuations, but to be negligible in the declination component. Thus, stations H and J were placed at opposite coastlines to achieve the greatest time difference between them for the passage of eclipse totality. The previous array studies have also shown that magnetic substorms and similar events occur near-simultaneously across the area, but that the quiet daily variation moves across with local solar time.

Thus, in Figs 2*a* and 2*b* one may expect to see: a background quiet daily variation, with station H lagging behind station J by 38 min; possibly some substorm activity occurring simultaneously at H and J; and an eclipse signal moving rapidly from H to J. In Fig. 2*c*, where the traces are subtracted, the effect of the substorm activity should disappear to first order, and one may expect to see the daily variation subtracted from itself 38 min out of phase to give a smooth curve of amplitude approximately 5 nT, and some interference effect between the eclipse signals at H and J. It is significant that the activity starting at 0711 UT has indeed largely cancelled itself out in Fig. 2*c*.

Fig. 2 *a*, The declination record for site H. *b*, The declination record for site J. *c*, H-record minus J-record. All records are plotted relative to arbitrary zeros. Amplitude conversion factors are 6.4 nT per minute of arc for station H, and 6.8 nT per minute of arc for station J.



The signal causing a depression in the declination traces at about 0638 UT is of interest as a possible eclipse effect, because: (1) it occurs at the time of the eclipse; (2) it does not cancel itself out in Fig. 2*c* as does the activity of 0711 UT; to be so strongly non-uniform it must be of local origin; and (3) it is of the correct sign, and order of magnitude, to be expected for the effect of an eclipse[2]. Several aspects inconsistent with a simple eclipse model should be pointed out, however: (1) the signal at 0638 UT is not of the same strength at stations H and J; (2) it has not moved from west to east at the known rate of the centre of eclipse totality on the ground; and (3) it starts gradually but ends abruptly.

Digitisation of the records obtained at the other stations will be undertaken and a more comprehensive report is planned.

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Magnetic superleaks

Two new natural materials which behave as superleaks to superfluid ^{4}He and also have magnetic properties are described here. In the search for magnetic superleaks the first choice was, obviously, a highly magnetic material such as a manufactured ferrite which has the advantage that its porosity is very uniform. We tested such a material but apparently not at a low enough temperature to be able to detect any onset point for the superfluid ^{4}He. The pores or gap distances must be extremely small[1]. Second, natural magnetic materials, among which the most obvious were different types of the so-called magnetites, were tested. Two magnetites of apparently quite different origin have been tested with superfluid ^{4}He. One from the south-east of Argentina, Province of Rio Negro about 41° S latitude and about 30 km from the Atlantic Ocean (sample A) and another of unknown origin taken at random from the Mining Department of the University of Delft (sample B). An X-ray diffraction analysis of these two samples gave the following components: For sample A, haematite (Fe_2O_3) (ferromagnetic), magnetite (Fe_3O_4) (ferrimagnetic), mica and garnet [$(Mg,Fe)_3Al_2(SiO_4)_3$] (paramagnetic) and for sample B, mainly magnetite (Fe_3O_4) (ferrimagnetic) and chlorite, a sheet silicate [$(Mg,Fe)_5AlSi_3O_{10}$]$(OH)_8$ (paramagnetic). Both samples are anisotropic aggregates and the magnetic characteristics are given at room temperature.

The method already described[2] to determine the onset temperatures of superleaks was used. A carbon sensor as a detector of the superfluid rests on the surface of a flat superleak inside a cell. When the superfluid through the superleak finally reaches the sensor, a noticeable jump in its resistance value is noted. This method has the advantage over the capillary method that no direct observations of the liquid helium level are necessary, difficulties inherent in an optical cryostat.

The obtained onset temperatures are: for sample A 1.57 K and for sample B 1.72 K. Applying the well-known expressions[3] of Ginzburg and Pitaevskii and of Mamaladze, the values for the gap width were obtained, respectively: for sample A, 36 Å and 24 Å and for sample B, 42 Å and 29 Å. Note that for superleaks made of natural materials the values of the onset temperatures are only approximate since they vary from different samples of the same material because of the non-uniformity of the diameters of the pores or gap widths. Also, with these measurements, there is a real doubt that the superleak is really a superleak. For instance, the way in which the superleak is fixed to the system, whether it is soldered to the glass of a cell or fixed with an indium wire gasket, might present microscopic leaks. Also it is impossible to apply any kind of reliable leak detection to a superleak. It is, however, possible to overcome that doubt with a reasonable number of observations and, as far as possible, mounting and dismounting the same sample to observe the reproducibility of the measurements.

The search for magnetic superleaks had its origin in the anisotropic magnetic superfluid phases of liquid ^{3}He recently found. The application of magnetic superleaks to the peculiar anisotropic magnetic superfluid phases of liquid ^{3}He at very low temperatures[4,5] might be relevant. However, to check magnetic superleaks in the anisotropic superfluid phases of ^{3}He will not be easy. In that physical situation those phases would be influenced by an adequately magnetised finite geometry medium, not necessarily those described here, which they filled, since below the transition temperatures the liquid has the properties of a superfluid and has magnetic properties, compared with those of nematic liquid crystals.

I thank Dr A. J. Leggett for valuable and encouraging remarks about the potential implications of a magnetic superleak in the superfluid phases of ^{3}He. Thanks are given to Dr R. A. Kühnel for the X-ray diffraction analysis and to Mr J. G. Krom for helping with the measurements.

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Towards a theory of stochastic vorticity-augmentation

THE mechanism of genesis of atmospheric tornado-like vortices is not yet known, although the hydrodynamic mechanism of a mature tornado is reasonably well understood thanks to recent research efforts. This lack of understanding of the physics of tornado genesis has been a great handicap in attempts to avert tornado occurrences. I present here a new hypothesis to account for the formation of tornadoes.

The hypothesis is based on the observation that thunderstorm associated tornadoes almost invariably occur in the environment of an intensely (vertically) sheared horizontal wind, say $V_x(z)$ which is penetrated by a vertical columnar jet-draught along the z-axis. It is easily recognised that the vertically sheared wind field could serve as an abundant source of ambient vorticity; nonetheless a casual theoretical consideration[1] of such a wind-jet interaction does not lead to a net gain of axial vorticity (ω_z) for the vertical jet at the expense of the horizontal vorticity ($\omega_y = -\partial V_x/\partial z$) according to the Helmholtz vorticity transport equation:

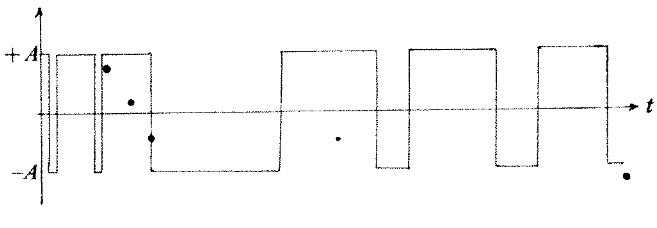
$$\frac{d\omega_z}{dt} = \omega_x \frac{\partial V_z}{\partial x} + \omega_y \frac{\partial V_z}{\partial y} + \omega_z \frac{\partial V_z}{\partial z} \simeq \omega_y \frac{\partial V_z}{\partial y} \qquad (1)$$

assuming $\partial/\partial z \ll \partial/\partial y$. A closer examination of the present wind-jet interaction, taking into account the resulting random lateral deflections of the jet caused by the alternating vortex sheddings in the wake of the cross-wind over the jet[2,3], shows that random sharp velocity gradients ($\partial V_z/\partial y$) are induced at the boundary of the random impulsively oscillating jet. This random input ($\omega_y \partial V_z/\partial y$) in equation (1) converts the latter into a stochastic differential equation having the axial vorticity (ω_z) as the output function. It is this stochastic vorticity transfer process that is considered herein as primarily responsible for starting a columnar vortex leading eventually to a mature tornado vortex after the pendant funnel extending down from the cloud base to the ground.

In this present study of the wind-jet interaction, I have constructed an extremely simplified model with only bare essential physics. For instance the entrainment which accounts for the incorporation of the ambient air into the jet is not taken into account. The hydrodynamic problem here is the determination of the probable increments of the jet axial vorticity due to the induced random input ($\omega_y \partial V_z/\partial y$) as a consequence of the random lateral displacement of the jet which can be viewed as a beam in Brownian motion[4]. Recognising the filamentary structure in a vortex jet[5] and even in the fine scales of turbulent flows[6], we envisage the jet as a bundle of vortex filaments along the jet axis (z) which faces a vertically sheared horizontal wind along the x-axis thus shedding alternating vortices on each side and, in so doing, inducing alternating hydrodynamic Magnus forces (along y-axis) on the jet. It can be shown that this many-filament system constitutes essentially a system of coupled nonlinear oscillators[3], under the influence of a high-frequency harmonic forces, which therefore oscillates as though a random force were acting on it[7,8]. The present many-body system is subdivided into a subsystem, namely a filament, and its environment. The influence of the environment on the subsystem is treated in a way similar that of a heat bath of statistical mechanics except that instead of a random force[9] we have random vorticity impulse with stochastic properties supposedly given. In this way the equation governing the growth of axial vorticity for the total system is reduced to that for the subsystem alone with the quantity ω_z in equation (1) representing an unnormalised axial vorticity at a sample point (o) of the test filament; the quantity $(\omega_y \partial V_z/\partial y)_o$ an effective random vorticity impulses at the point o. We have thus a one-dimensional model neglecting the feedback and damping of the vortex-jet disturbances.

Consider a surface velocity gradient $(\partial V_z/\partial y)_o$ depicted by the simplest plausible stationary function known as the random telegraphic signals with values $+A$ and $-A$ alternately as shown in Fig. 1 where $A\alpha(t) = \omega_y(\partial V_z/\partial y)_o$ and $|\alpha(t)| = 1$. The probability of switching over the signal from one value to its

Fig. 1 Sample function of the $(+A, -A)$ random telegraph signal $(A\alpha(t), t > 0)$



opposite is assumed to be governed by a Poisson distribution at a mean rate of $\mu\ s^{-1}$.

The incremental axial vorticity can be obtained from equation (1):

$$\Delta\omega_z(\tau) = \omega_z(\tau) - \omega_z(0) = A\int_0^\tau \alpha(t)\,dt \qquad (2)$$

The ensemble average of the squared incremental axial vorticity is given by[10]

$$\langle[\Delta\omega_z(\tau)]^2\rangle = A^2\tau \int_{-\infty}^{\infty}\langle\alpha(o)\alpha(s)\rangle\,ds \qquad (3)$$

the integrand $\langle\alpha(o)\alpha(s)\rangle$ denotes the correlation function of the stationary random function $\alpha(t)$ and is equal to $\exp(-2\mu|s|)$. Thus:

$$\langle[\Delta\omega_z(\tau)]^2\rangle = \omega_y{}^2(\partial V_z/\partial y)_0^2\tau\,\mu^{-1} \qquad (4)$$

which gives the important parameter depicting the stochastic growth rate of the columnar axial vorticity of a vortex filament of the jet.

The more detailed probabilistic information is given by the probability $P(\Delta\omega_z, t)d(\Delta\omega_z)$ that the increment $\Delta\omega_z$ at time t lies between $\Delta\omega_z$ and $\Delta\omega_z + d(\Delta\omega_z)$. Recognising the Markovian nature of the stochastic process herein, we can determine the probability density $P(\Delta\omega_z, t)$ by solving its governing equation in the form of a driftless Fokker-Planck equation for diffusion[9] and obtain the Gaussian distribution:

$$P(\Delta\omega_z, t) = (4\pi Dt)^{-1/2}\exp[-(\Delta\omega_z)^2/4Dt] \qquad (5)$$

where $D = \langle(\Delta\omega_z)^2\rangle/2\tau$. If a critical value for $\Delta\omega_z$ is prescribed, say $(\Delta\omega_z)^* = \beta\sqrt{\langle(\Delta\omega_z)^2\rangle}$, for large β,

$$P[\Delta\omega_z > (\Delta\omega_z)^*] \simeq (2\pi)^{-1/2}\beta^{-1}\exp(-\beta^2/2) \qquad (6)$$

In conclusion, a new cause of vorticity transfer between an ambient sheared-wind and a transverse-penetrating jet is proposed for which an elementary one-dimensional theory is formulated. It points out the relevant quantities to be determined, theoretically or experimentally, in describing the present stochastic mode of vorticity-augmentation.

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Theoretical evidence for metastable cyclic ozone

OZONE is a vital component of the atmosphere. Knowledge of its properties is important to the understanding of its involvement in chemical and photo-induced reactions which can affect the stability of the ozone concentration in the upper atmosphere. The reactivity of ozone and its unusually low thermochemical stability with respect to partial dissociation are difficult to reconcile with an apparently multiply bonded symmetric bent structure. This has led to speculation[1,2] concerning alternative forms of ozone such as the equilateral triangular cyclic form (isoelectronic with cyclopropane) from which facile partial dissociation could be envisioned. To have an important role such a form would presumably need to be thermally accessible from the ground state and bound with respect to partial dissociation. The spectroscopic structure determination of the symmetric bent ground state[3–10] has been finalised[10] (r_e (O–O) 127.1 pm, angle 116.8°), and there was recently experimental confirmation[11,12] of excited states of this species[13–16,22] which are bound with respect to dissociation, including the predicted $^3B_2$ state[13–17,22]. Investigations of bound excited states which are stable to partial dissociation have been stimulated by the sequential observation during O_2 radiolysis experiments[12,18,19] of two short-lived 'ozone precursors' $O_3{}^\alpha$ and $O_3{}^\beta$ following recombination of ground state O_2 and O. $O_3{}^\beta$ may be vibrationally excited ground state ozone[20], whereas the $O_3{}^\alpha$ precursor which shows absorption[12] at ~ 315 nm in the ultraviolet (and possibly[21] the emission observed at $1500\ cm^{-1}$ in the infrared) has not yet been given an identity beyond an apparent lifetime of 5 µs. The species showing the single infrared emission[21] is interesting as D_{3h} cyclic ozone is expected to show one (degenerate) infrared active fundamental, fewer than any alternative structure. Theoretical evidence for the stability of cyclic ozone has been conflicting. In 1973 Wright[1] calculated it to be 26 kJ mol^{-1} more stable than the ground state; in 1972 and 1975 Hay *et al.*[13,[illegible]2] calculated it to be less stable by ~ 150 kJ mol^{-1} (that is, above partial dissociation energy $\Delta H^\circ = 101$ kJ mol^{-1}) and in 1974 Shih *et al.*[14] have concluded it is 63 kJ mol^{-1} above the ground state. Shih *et al.*[14] have recognised that considerable uncertainty still exists in their computation on total and relative energies, and their calculated ground state structural parameters differ significantly from experiment. We have reinvestigated the relative stability of cyclic ozone and symmetric bent ozone and have obtained a total energy for the ground state of −224.7710 hartree which is 471 kJ mol^{-1} lower than the previous best model wavefunction of Hay *et al.*[13] who predicted cyclic ozone to be above dissociation. In other words, the relative shortcoming in total energy in all previous computations on each conformer has been around five times the key energy difference between ground state ozone and the partial dissociation products, $\Delta H^\circ = 101$ kJ mol^{-1}. The computed optimum geometric parameters (bond length $r_e = 126.4$ pm and bond angle $\alpha_e = 117.3°$) are sufficiently close to the experimental values to lend some confidence to our model wavefunctions in regard to structural predictions. The corresponding wavefunction for cyclic ozone has an energy expectation of −224.7637 hartree at an optimum bond length of $r_e = 143.5$ pm (bond angle 60°). On this evidence the difference in the energy of the local minima on the ozone ground state conformation hypersurface is less than one fifth of the partial dissociation energy. We, therefore, confidently predict the existence of a bound species of ozone having an equilateral triangle conformation. We believe this species may be the $O_3{}^\alpha$ precursor, which in the experiments referred to[18,19] is presumably vibrationally excited.

Our disagreement with Hay *et al.*[13] regarding the stability of the cyclic conformer has a number of origins. Hay *et al.* seem not to have optimised molecular geometries using their best bases and their best correlated wavefunctions, and have apparently omitted polarisation basis functions in any study of the cyclic conformer. The basis sets we used are comparable to the best previously used at the Hartree Fock level E_{HF} (bent, SCF optimised geometry) −224.314 hartree, E_{HF} (bent, CEPA optimised geometry) −224.3058 hartree and E_{HF} (cyclic, CEPA optimised geometry) −224.3011 hartree. The significantly lower final conformational energies we have obtained despite the fact that our basis is not designed to obtain inner 'atomic' correlation effects is due to the use of a pair natural orbital (PNO) basis which is designed to optimise the configuration expansion convergence. We used ~ 750 PNO configurations in our final calculations on each conformer, which is equivalent to 1–2×10^4 canonical configurations based on HF orbitals.

Our computer modelling of the wavefunction of each ozone species was intended to reduce previous basis set and geometry-optimisation limitations, taking the following course:

(1) Initial optimised geometries for each species were obtained from a survey of the surface (1014 points)[23] using simple independent particle SCF wavefunctions based on atomic STO-3G basis augmented with sets of single component gaussian bond functions between each pair of oxygens (3 sets). The bond function sets comprised one *s* gaussian and three *p* gaussians all of which were single gaussian fits to corresponding Slater functions of exponent $\xi = 1.1$. These bond functions afforded some valence deformation and polarization.

(2) With these geometries, large and flexible atomic oxygen basis {(9*s*, 5*p*) → [5, 1, 1, 1, 1; 3,2] in standard notation; ref. 24} were situated at each nucleus, and three equivalent sets of mid-bond bond functions for each species were optimised at the SCF level by varying the characteristic Slater exponent for the sets (ξ_{opt} (ground state) = 1.87, ξ_{opt} (cyclic) = 1.95).

(3) With the partially optimised set of 45 expansion functions so obtained (data to be published, P.G.B.) for each species, the valence SCF molecular orbitals were subject to a localisation procedure and the many-body effects of electron correlation among the valence electrons was estimated by following the CEPA–PNO procedure of Meyer[25] and Ahlrichs *et al.*[26]. At this stage correlation interaction between pairs only belonging to the same atom as well as the intrapair correlation were taken into account (yielding 57 sets of pair natural orbitals).

(4) Using the incomplete CEPA–PNO wavefunctions, geometric parameters were reoptimised (small changes resulted) yielding the values quoted above.

(5) Full valence CEPA–PNO correlation energy corrections were obtained for each reoptimised geometry species (including 81 sets of pair natural orbitals) yielding the respective energies for the ground state and cyclic ozone quoted above. The CEPA–PNO energy difference of 19.2 kJ mol^{-1} favouring symmetric bent ozone is paralleled by the corresponding PNO–CI[25,26] difference calculated by us of 15.8 kJ mol^{-1}.

We believe that limitations in our model due to incomplete basis sets and neglect of core and core–valence correlation will affect our computations of relative energies by less than 20 kJ mol^{-1}, and that further refinement of the wavefunctions for each species will not affect our prediction of the existence and stability of cyclic ozone.

We expect cyclic ozone to be involved in ozone reaction kinetics and atmosphere chemistry where inclusion of its role may improve atmospheric modelling[27]. In interstellar gas clouds such a metastable species might be expected to be trapped by slow collisional deactivation. The absence of a permanent dipole moment, however, is against the usual methods of detection of pure rotational transitions which would provide the best confirmation of the identity of cyclic ozone.

We are working to calculate the spectroscopic and other properties of cyclic ozone.

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High absorption efficiency for ingested plutonium in crabs

ALTHOUGH marine organisms have been strongly implicated in the biogeochemical cycle of plutonium, there is disagreement as to whether they can assimilate it efficiently[1–7]. We have examined the fate of plutonium in a single, worm-crab food chain after ingestion of the isotope which had been metabolically incorporated into the predator's food. Contrary to absorption efficiencies of tenths to hundredths of 1% derived from studies with vertebrates[8–10], our results show that relatively large fractions of plutonium are readily absorbed from ingested food and incorporated into the predator's tissues.

The radioisotope ^{237}Pu was used in all experiments. The advantages of using ^{237}Pu tracer to study environmental kinetics of ^{239,240}Pu have been discussed by others[5,11]. The relatively high specific activity of this isotope facilitates the use of amounts of plutonium similar to the levels found in the environment. Tracer solutions were prepared by adding sufficient ^{237}Pu in either the +4 or +6 oxidation state to 50 ml cotton-filtered seawater to give a concentration of approximately 8 nCi ml^{-1}. Polychaete worms (*Nereis diversicolor*) were exposed to labelled seawater for several days, thoroughly rinsed and whole-body counted for ^{237}Pu activity as described previously[5]. In the first series of experiments six individual shore crabs (*Carcinus maenas*) received two to three contaminated *Nereis* and were whole-body counted immediately, replaced in their individual aquaria with running seawater (≃18 °C) and given sufficient unlabelled worms to ingest throughout the experiment. The crabs were whole-body counted periodically to measure excretion of the radionuclide, and at the same time their faecal pellets were filtered from the water and monitored for ^{237}Pu to assess defaecation as a route of elimination. At prescribed times, individuals were dissected to show the distribution of residual ^{237}Pu. In a second series of experiments, two large edible crabs (*Cancer pagurus*) ingested four separate rations of labelled worms within 48 h and were subsequently maintained for 3 weeks in conditions similar to those already described. The size of *Cancer* prohibited whole-body counting and the elimination was estimated from the knowledge of ^{237}Pu ingested, lost by defaecation and retained in the various tissues.

Figure 1 shows the plutonium whole-body elimination patterns displayed by *Carcinus*. The initial rapid loss noted during the first 2–4 d represented gut clearance of unassimilated plutonium. This was verified by analysis of faecal pellets; during this period approximatety 90% of whole-body loss occurred through defaecation. The fraction of total loss due to defaecation then decreased markedly from 90 to 40%, suggesting the increasing importance of soluable excretion processes. This period corresponded to the phase during which whole-body elimination proceeded at a much slower, nearly exponential rate (solid lines in Fig. 1). This phase represents loss of that fraction of plutonium absorbed from the food; extrapolation of this component to day 0 facilitates estimation of absorption efficiency. The high values for the six individuals, from about 20–60%, were not expected in view of results from previously published vertebrate studies[8–10], and we are unaware of comparable data for invertebrates.

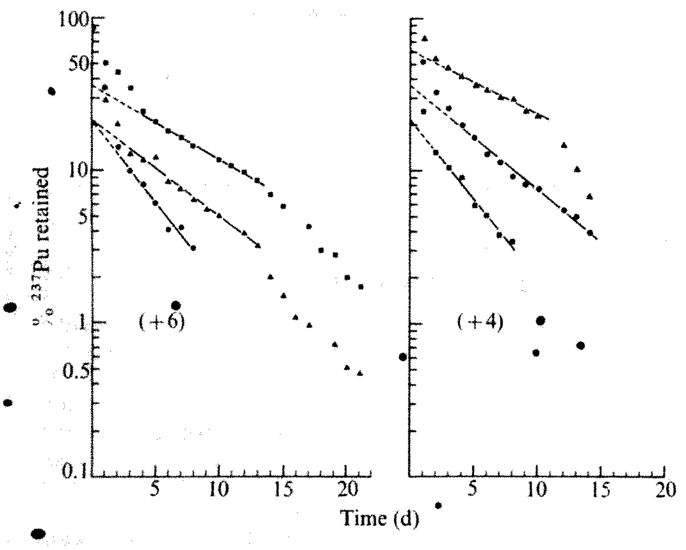

Fig. 1 Elimination of ^{237}Pu after single ingestion of labelled worms by six individual *Carcinus*. Assimilation coefficients for Pu were estimated by resolving the different components of the loss curves using standard mathematical methods routinely used in radioecological studies[12–15]. The loss curves are treated as a two component elimination system described by the expression $A_t = A_0(a_1 e^{-k_1 t} + a_2 e^{-k_2 t})$, where A_t is the whole-body radioactivity at time t, and A_0 is the initial whole-body radioactivity immediately following ingestion of labelled food. a_1 represents the fraction of unassimilated ^{237}Pu eliminated at a rate k_1; a_2 is that fraction of assimilated ^{237}Pu eliminated at rate k_2; a_2 (absorption efficiency in our study) is determined graphically by extrapolation of the linear part of the long component (solid line) to time zero. Enhanced elimination of the assimilated ^{237}Pu noted in three individuals after about 12–14 days is assumed to be a reflection of increased metabolic rate probably associated with a given stage of the intermoult cycle; therefore these data were not included when fitting the longer component by a least squares estimate.

Dissections indicated that the plutonium absorbed from the food had become distributed within the crabs. In most cases the largest fraction (43–85%) was in the hepatopancreas which serves as the digestive gland. Lesser amounts were in other tissues such as shell (8–43%), and in muscle and gill (5–10%). ^{237}Pu was at or below the detection limit in stomach, gut and haemolymph. In the second experiment a larger crab, *Cancer*, was given more ^{237}Pu to facilitate measurement. Because of the experimental design it was not possible to estimate absorption efficiency; however, it was undoubtedly high, for 12 and 41% of ingested +6 and +4 ^{237}Pu, respectively, was retained in the tissues at the time of dissection. The relative distribution of the radionuclide among the tissues of *Cancer* generally confirmed that noted with the shore crabs.

Concentrations (c.p.m. per g wet mass) of residual ^{237}Pu in both crabs were by far the highest in hepatopancreas, with less in gill, stomach, shell and muscle. Certain trace elements[16] and radionuclides[17] are absorbed from ingested food and can accumulate to high levels in crustacean hepatopancreas; similar mechanisms seem to operate in the initial absorption of the large fraction of plutonium removed from the crab's food during passage through the gut. The low concentration of Pu in muscle relative to the other tissues was similar to that found in crabs and lobsters which either had been contaminated in the environment[2,18] or had accumulated the radionuclide directly from water in laboratory studies[19].

Reports[1,7,20] have emphasised the need for more information on transuranic transfer coefficients between various links in marine food chains to predict total amounts potentially available to man. We have examined this aspect in a simple, two-step invertebrate food chain and have found that Pu naturally incorporated into food can be assimilated into the predator's tissues with efficiencies several orders of magnitude higher than those characteristic of mammalian systems. The difference in absorption efficiencies between mammals and marine crustaceans is most likely a result of differences in digestive physiology; it is, however, conceivable that the way the radionuclide was administered influenced the degree of assimilation. Although only data for crabs are reported here, similar experiments carried out in our laboratory suggest that high plutonium absorption efficiency is also common to other marine invertebrates. Thus, it is possible that data indicating extremely low gastrointestinal absorption of plutonium by vertebrates are not applicable to species comprising the bulk of the marine biomass.

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When is sex environmentally determined?

THERE are several sex determining mechanisms which produce two sexes in a population (dioecy or gonochorism)[1–5]. In many of these, the offspring's sex is determined at or before conception, as in male or female heterogamety. In several organisms, however, the offspring's sex is determined later than conception, by some environmental influence upon the offspring. We consider here why these environmental mechanisms have been maintained; that is, why have they not been replaced with an alternative system that determines sex at conception? We propose that labile sex determination (not fixed at conception) is favoured by natural selection when an individual's fitness (as a male or female) is strongly influenced by environmental conditions and where the individual has little control over which environment it will experience. Our argument also applies to sex expression in hermaphrodites[6,7].

The basis of a theory of labile sex determination lies with two principles, first noted by Fisher[8], who applied them to sex ratio evolution: (1) both sexes contribute autosomal genes equally to the next generation, that is, everyone has a mother and a father, and (2) the fitness of an individual is measured relative to others of the same sex. Relative fitness is the ratio of an individual's actual fitness (survival × fertility) to the average fitness in that sex and must account for the relative abundances of both sexes. Selection thus favours the ability of a parent or offspring to manipulate the sex ratio to result in offspring with the highest fitness in each sex[9].

To illustrate, if an individual finds itself in an environment where it can become a below average female or an above average male, selection will favour its becoming male because it can pass on more of its genes than if it were female. Selection may therefore favour 'environmental sex determination' (ESD) because of the control it allows an individual. When sex is determined at conception, the individual no longer has this control but can begin 'developing into' its sex immediately. Early development of sex allows an individual to become a better male or female and is probably the major advantage of genotypic sex determination.

Selection should most strongly favour ESD when (1) the offspring enters an environment, away from the parent, which has a large effect on its lifetime fitness; this environment is patchy, some patches conferring more of an advantage to females than to males and vice versa; and (2) the offspring and parent have little control (or predictive ability) over which patch type the offspring enters. These conditions make it unfavourable to determine sex at conception because of the possibility that a male will enter a patch that is much more favourable for a female, or the reverse.

There are several ways in which an environment may be 'patchy' relative to the selective value of being a male or a female. (1) There is local competition for mates. If an area has a high concentration of females and a low concentration of males, then an individual enjoys the greatest success when it is a male. (2) Resources may have differential value to males or females, and they may be patchy in distribution. If an offspring must garnish most of its resources in a restricted environment, and if there is a sex difference in the advantage of the resource, then the offspring should become the sex which benefits the most under the circumstances. Resources which may have differential value to sex function include sunlight (in plants) and other nutrients for growth (a large size is often more advantageous to one sex than the other). (3) Predation (or other mortality sources) may be patchy and could be sex specific.

The inability of the parent or offspring to choose a patch type also has a major role in favouring ESD. If an offspring can choose its patch, sex can be determined at conception and the offspring enters the patch that will give its sex the highest fitness. Inability to choose patch type will often be correlated with immobility; however, parental mobility does not preclude ESD. If parents deposit offspring in (or offspring enter) environments from which they cannot escape during some crucial stage of development, selection may favour an environmental mechanism when the parent or offspring is unable to evaluate the patch type prior to entry. In contrast, if the parent can evaluate the patch (but the offspring cannot leave it), selection may favour parental ability to match the sex of the offspring to the appropriate patch. Such mechanisms occur among parasitic Hymenoptera[10,11].

Environmental sex determination is known in several plants and animals, and these seem to fulfil the expected characteristics.

Many species of nematodes which parasitise insects (family Mermithidae) and some which parasitise plants (genera *Meloidogyne* and *Heterodera*) are believed to have ESD[2,12–14]. The life histories are basically alike: young (eggs) are either deposited on a host or they locate a host and enter. The larvae attain almost adult size while in the host. Adults (at least males) leave and mate outside the host[12,15].

The sex of the nematode is determined after entering the host, and sex ratio varies with parasite density inside the host. High density results in an abundance of males, whereas low density results in mostly females[2,12–14]. Large size is apparently more important to female than to male fitness (females are universally the larger sex in nematodes). Thus a juvenile in a crowded host will not be able to attain a large size; it can become an average male or a below average female: selection should favour it becoming the male. In uncrowded hosts, an individual gains more as a female.

Among marine invertebrates, ESD is known in *Bonellia* (an echiurid), and several parasitic isopods (for example, *Stegophryxus* and *Ione*)[2,3,16]. These organisms usually have dwarf males which live on the female. Larvae are planktonic and adults are immobile (*Bonellia*) or are restricted to the host. Larvae that settle alone become females, those that attach to females become males. In these, the resource of strongest sex-dependent value is the female, because of the ability of the male to remain small, live upon her and fertilise her eggs. The example of parasitic males partially violates the condition that the organism be unable to choose patch type. A larva that encounters a female can probably choose to avoid settling on her. Despite this, the absence of choice still largely applies because all larvae presumably do not encounter females (or do not have the choice to). Therefore, a larva that differentiated into a male too early would not be guaranteed to encounter a female.

Sex may also be environmentally determined in monstrillid crustaceans[12]. These parasitic copepods have life cycles similar to the nematodes discussed above in that the adults are free and mobile, and most of the larval growth takes place inside the host. As in nematodes, crowding in the host results in a preponderance of males.

Among higher plants, there are numerous examples of dioecious species where some individuals change sex between seasons of reproductive activity[4,5,17]; this provides evidence for the widespread occurrence of ESD in a group where it was previously given very little attention[18,19]. Also, there are many cases where male and female plants occupy different habitats, suggesting ESD or perhaps differential mortality by sex[17].

Several well-documented examples of plants with ESD are orchids of the genera *Cycnoches* and *Catasetum*[20,21]. Sunlight seems to be the resource which is differentially beneficial. Plants grown in bright sunlight become female, those grown in shade become male. Also, larger plants tend to be female. It is not clear why sunlight is more important to femaleness than to maleness (why should it be more important to seed production than pollen production?), but the pattern is well documented.

The arguments have been thus far directed towards maleness against femaleness in dioecious species. Natural selection should also favour the ability of a hermaphrodite living in a patchy environment to alter its allocation of resources between male and female function[7].

A sequential hermaphrodite should exercise control over the time at which it changes sex in accord with the above idea. As fitness of the second sex is often size dependent, it should often change sex according to size rather than age. For example, if an individual is reduced in size just before the expected size of sex change, it should remain the first sex until recuperated from the perturbation. Examples of this are well documented: in a polychaete (*Ophryotrocha puerilis*)[2] and a plant (*Arisaema triphyllum*[4,5,22,23]. Other examples of labile sex change, related to male against female fitness, are also known in fish (the labrid, *Labroides dimidiatus*)[24] and some marine gastropods (for example, *Crepidula*)[2,25].

In a simultaneous hermaphrodite, selection should favour the ability to alter the ratio of sperm (pollen) to ova (seeds) produced in response to environmental conditions[7] (as simultaneous hermaphroditism is commonly associated with immobility in the adult stage)[6]. Examples seem to be scanty, but the reallocation in monoecious plants due to factors such as nutrition or sunlight is well documented[4,5,26,27] (C. Smith, personal communication), particularly in the Russian literature[28].

Interestingly, this suggests that one selective advantage of monoecy in plants is that it allows the use of hormonal systems to control male against female allocation. This alteration is as simple as changing the ratio of male to female flowers. Supporting this idea is the observation that populations consisting of males, females and monoecious individuals are among the most common of all types of mixed populations in plants[28]. We interpret the males or females as hermaphrodites who have reallocated resources to one or the other sex. Such lability would seem to be less possible in plants with hermaphroditic flowers.

As this paper was being written we became aware that similar ideas were being developed in four other labs: K. E. Hoagland with protandrous molluscs; C. Smith for monoecious trees; L. Gilbert and M. Condon for protandrous vines; and C. Freeman, K. Harper and L. Klikoff for dioecious plants. We acknowledge their independent thought. We thank G. C. Williams, R. Shine, J. Cranford, J. Werren, N. Negus, P. Berger, G. H. Orians, J. Maynard Smith and Thomas Gibson for cooperation. This work was supported by a grant from the PHS.

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Monogamy and sex change by aggressive dominance in coral reef fish

In several sequentially hermaphroditic coral reef fish, of which individuals first function as females and then males (protogynous hermaphroditism), dominant males can control production of other males by aggressive dominance over females[1–4]. Robertson suggested that socially controlled protogynous sex changes might operate only in species with a well defined polygynous social system "based on individual relationships"[2,3]. Further exogenous and endogenous factors may control sex change in species which school anonymously and in which the individuals lack special individual relationships. We describe here socially controlled proteandric hermaphroditism (individuals functioning first as males and then as females) in the anemone fish *Amphiprion*, in which females control production of females by aggressive dominance over males.

Amphiprion is widely distributed in the tropical and subtropical Indopacific region and is symbiotic with several sea anemones[5]. A 38-month field study of the ecology and social behaviour of *A. bicinctus* in Eilat, Israel (Red Sea) and a 2-month study of *A. alkallopisos* at Aldabra indicated that the breeding population of both consisted of large functional females with smaller functional males (Table 1). Gonadal examinations of 170 *A. alkallopisos* and 41 *A. bicinctus* showed that both species are born as males and females, but during ontogeny undergo functional male then female phases. Immature oocytes are always present in the testes of functional males (Fig. 1*b*), while functional females show no sign of testicular tissue.

Table 1 Mean and standard deviation of body length and wet body weight of the 10 largest males and females of *A. akallopisos* and *A. bicinctus*

	A. akallopisos		*A. bicinctus*	
	Male	Female	Male	Female
Length (mm)	73±3	97±5	113±7	129±6
Weight (g)	7.1±1.1	19.5±2.9	28.0±3.9	46.1±7.6

The typical social unit is a large female, a single smaller male and a varying number of subadults and juveniles, none of which is offspring of the adult pair. Both species

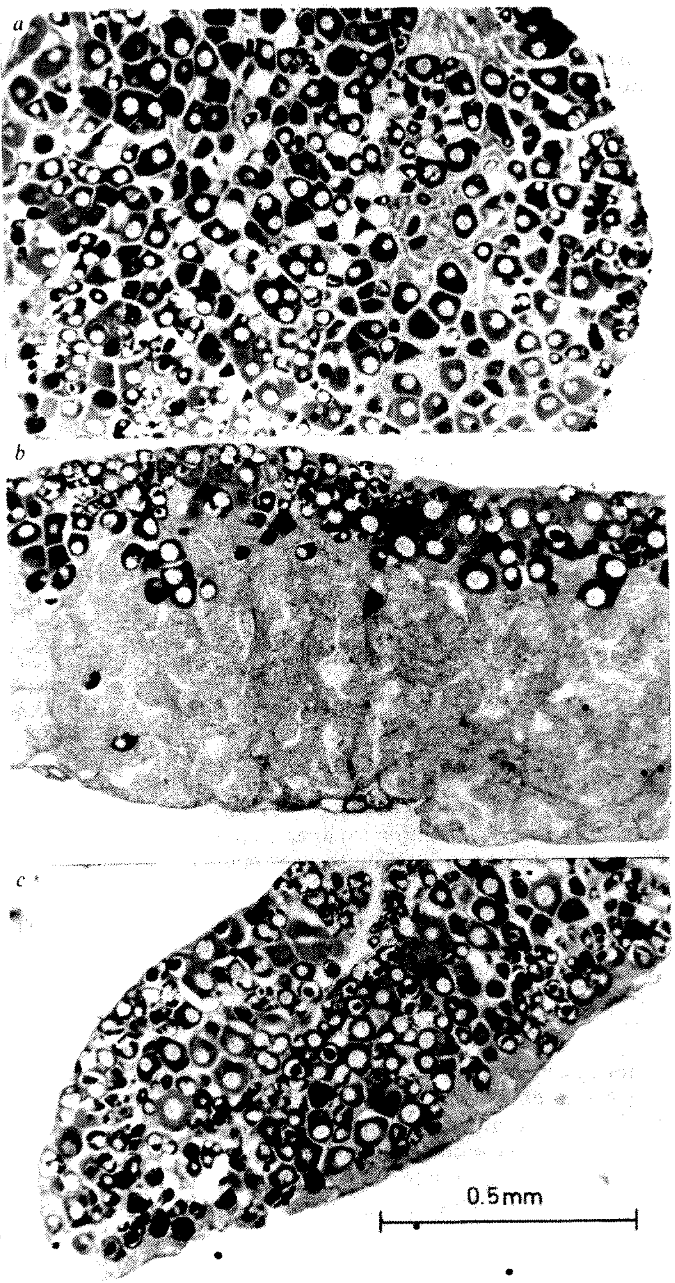

Fig. 1 Development of the testes of *A. akallopisos* according to the social status of the male. Males of approximately the same body length were selected. *a*, Previous beta-male (65 mm) 63 d after removal of the dominant female; *b*, β-male (70 mm) in functional state; *c*, γ-male (68 mm), development of mature testicular tissue suppressed.

live in closed monogamous groups based on individual recognition and individual pair bonding[6,7]. Several pairs of *A. bicinctus* remained on their home anemone for 3 yr without changing partners. Differences in group size result from differences in the ecology of occupied anemones (Table 2). Occasionally groups of up to 10 adult *A. akallopisos* are found on a single anemone colony. The average group size of *Amphiprion*, however, is restricted by the dimensions of the anemones to no more than two adults. "Monogamy by force" and permanent pair formation is favoured under these ecological limitations[7]. The largest and oldest individual of a group is always female and dominates the male, subadults and juveniles (Fig. 2). Subadult males are attacked by both sexes, the dominant alpha-female and high ranking males (Fig. 2). The beta-male particularly attacks the sexually competing gamma-male. In two groups of four *A. akallopisos* each, 53% of 861 and 64% of 559 observed, intragroup aggressive acts were performed by beta against gamma. The largest male prevents other males from spawning, thereby maintaining a monogamous social structure. The intragroup social pressure determines the gonadal development of the subdominants. Their testes are smaller and show little or no mature testicular tissue. Low ranking males are psychophysiologically castrated.

Sex reversal occurs in different social situations. After removal of a dominant female in an aquarium group of five mature *A. akallopisos*, the beta-male took the alpha-position and changed sex in less than 63 d (Fig. 1*a*). Sex change was also induced in field experiments by the removal of females from 24 pairs of *A. bicinctus*; six of the 24 males remained solitary while the remaining 18, joined by subadults of unknown origin, turned into females. The first of these females laid eggs after 26 d. To demonstrate the influence of dominance on sex change, different sized males were paired forcibly on empty anemones in the reef, and the gonads of six pairs were examined after 2 months and another 10 pairs after 6 months. The largest and dominant males always changed to females. Sex reversal also occurred in the absence of intragroup social pressure. Twelve functional males placed separately in the sea, six caged and six free, after 2 months showed various degrees of increased oocyte production. The uncaged males were more advanced towards becoming female in contrast to the caged males which were probably more stressed. Sex change was much slower in both experimental groups compared with dominant males which were paired forcibly with smaller males in the same conditions. The reversal of sex change from female to male could not be induced experimentally. Forcible pairing of functional females ($n=7$) resulted in death or severe injury to the subdominants. This study demonstrates socially controlled sex change by aggressive dominance. Females control the procreation of other females. They restrict the size of the breeding population and actively suppress males which are likely candidates for future females.

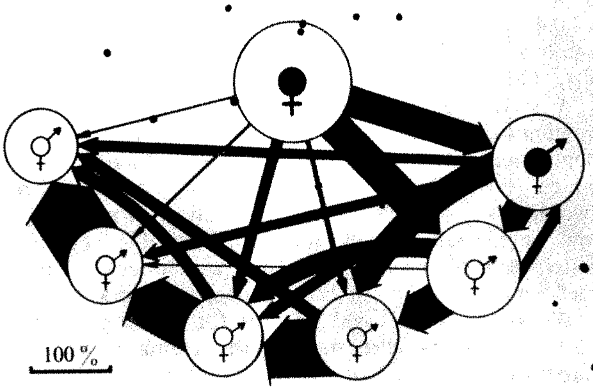

Fig. 2 Rank order by aggressive dominance in a group of seven *A. akallopisos*. An aggressive interaction is defined as a single chase, bite or frontal display. The relative body size of each individual is expressed as the square of the circle. The sex sign indicates the sex determination of the individual. A full circle denotes a functional, reproductive individual. The direction of the big arrows points to the receiver of the aggressive act. The distribution of aggressive acts of each individual is expressed as a percentage of total aggressive acts (= diameter of the arrow) which are performed by one individual against other group members. 752 intragroup aggressive acts were recorded.

If *Amphiprion* were gonochoristic (1 : 1 sex ratio), the chances for homosexual and heterosexual pairings resulting from randomly arriving larvae or juveniles or migrating adults would be equally distributed. Redistribution of sexually mismatched individuals would presumably result in migration of one of the individuals. Migration, however, increases the risk of predation. Furthermore, low population density and unpredictable distribution of the host reduces the chances of finding an anemone or an anemone with a partner of the opposite sex. These risks can be alleviated by socially controlled hermaphroditism where the largest and dominant animal controls the sex of the subdominants. In the case of death of a pair partner, this partner is easily replaced: the female through a fast sex change of the dominant male, the male from the stock of subadults or juveniles which arrive or live together with the adult pair. Juveniles are stunted but grow rapidly when an adult dies[5,7]. Furthermore, their ambisexual nature[8] allows formation of pairs by dominance when no adults are present. The young fish in the proximity of the breeding fish are always an insurance of potential males and females. Socially controlled sex change enables recruitment of breeding pairs independent of the sex of arriving young fish and without the necessity of leaving the host in search of a mate. Functional mates are produced whenever they are needed. It seems to be advantageous for a female to be larger than the male of the pair. A small *Amphiprion*-male can successfully fertilise and guard the eggs of a big female. Size increase of the male would not necessarily increase the reproductive success of the male. On the other hand, a larger female can produce more eggs than a smaller one (body weight and weight of ovaries are correlated in both species, *A. akallopisos* $r = 0.55$ ($P < 0.001$), *A. bicinctus*

Table 2 Comparison of both fish species and their host anemones

Species	Host anemones	Life style of host	No. of observed hosts	No. of adult fish per host	No. of juvenile fish per host	% Hosts occupied
A. akallopisos	*Radianthus ritteri*	Colonial	82 Colonies	2.6	0.6	100
A. bicinctus	*Gyrostoma helianthus*	Solitary	228	1.1	0.3	99.2
	G.quadricolor *R.koseirensis*	Solitary	122	—	0.9	69.7

The data for *A. akallopisos* were taken along transects in the lagoon channels of Pass Femme and Pass du Bois at Aldabra; for *A. bicinctus* in different reef zonations of the Nature Reserve in Eilat.

$r=0.45$ ($P<0.05$)). Therefore, the female of a monogamous pair benefits in reproduction by an increase in size. This could determine the direction of sex change.

A system of monogamy and sex change by aggressive dominance of females with them controlling the breeding population seems to be adaptive in ecological conditions which restrict group size to an average of not more than two adults. Probably the system evolved in response to the low population density and unpredictable, opportunistic distribution of the host anemones which occur in nearly all reef zonations from shallow to deeper waters.

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Behavioural divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* complex

STUDIES of the polytene chromosomes of the mosquitoes of the *Anopheles gambiae* complex have revealed fixed rearrangements based on paracentric inversions between all the six sibling species and inversion polymorphisms at the intraspecific level[1–5]. Inversion polymorphisms involve mainly chromosome arm 2R and are particularly frequent in the species formerly designated as A and B for which the names *An. gambiae* Giles, 1902 and *An. arabiensis* Patton, 1905 have been proposed[6]. These are the most ecologically adaptable members of the complex and the most important medically[7]. They are sympatric over the greater part of the African savannas. We report here on relationships between the behaviour of these mosquitoes and their inversion polymorphism.

Investigations were conducted in the Garki district (Kano State, Nigeria), a typical Sudan Savanna area located north-east of Kano where *An. arabiensis* generally outnumbers *An. gambiae* and breeding of both species is strictly seasonal, depending on rainfall. The main working hypothesis was that of a differential probability of contact with man and/or his domestic environment for the mosquitoes carriers of different inversion karyotypes. The availability of suitable polytene chromosomes in the adult ovarian nurse cells[8] enabled us to test this hypothesis directly by comparing inversion frequencies in female mosquitoes collected on the same day and in the same locality (a village in the Garki district) but in different situations (for example, indoors and outdoors). Preliminary evidence of non-random distribution of *An. arabiensis* with different 2R arrangements[9,10], obtained in 1972 and 1973, stimulated further comparative sampling in 1974 and 1975.

The 2R chromosome polymorphism of *An. arabiensis* from the Garki district involves six common paracentric inversions (Fig. 1). This complex system of overlapping and independent contiguous inversions permits no less than 15 different whole arm arrangements (2R, 2R*b*, 2R*bc*, 2R*d*, 2R*bd*, 2R*bcd*, 2R*a*, 2R*ab*, 2R*abc*, 2R*ad*, 2R*abd*, 2R*abcd*, 2R*be*, 2R*abe*, 2R*bf*), the frequencies of which cannot always be estimated because some of the 105 heterozygous combinations result in karyotypes which are very difficult to distinguish from one another (for example, 2R*a*/2R*bcd*, 2R*abc*/2R*d*, 2R*ad*/2R*bc*, 2R/2R*abcd*). Variation in 2R was therefore studied in terms of three sectional standards with the corresponding independent inversion systems $+^a \leftrightarrow a$, $+^b \leftrightarrow b \leftrightarrow bc$ and $+^d \leftrightarrow d$ and with two other overlapping inversions, *be* overlapping the last two systems and *bf* overlapping all three. As might be expected from close linkage existing between the independent inversions, obvious departures from random association were observed; these will be analysed separately.

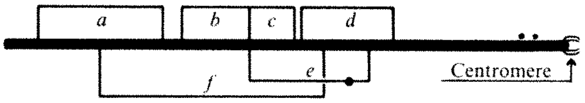

Fig. 1 Diagrammatic representation of 2R chromosome inversions in *An. arabiensis* from the Garki district. Inversion pairs *b-c* and *b-e* are interpreted as cases of extreme contiguity with one break point apparently coincident. The available evidence indicates that both *c* and *e* are based on 2R*b* as the inverted arrangements so far recorded are 2R*b*, 2R*bc* and 2R*be*, but not 2R*c* nor 2R*e*. Note that, in absence of 2R*b*, both arrangements 2R*bc* and 2R*be* could be interpreted as due to tandem inversions based on 2R. Inversion *f* is based on 2R*b* producing the 2R*bf* arrangement which overlaps with all other 2R inversions.

Table 1 compares the basic 2R chromosome variability in samples of *An. arabiensis* females collected in the village of Jirima during the 1973–1975 rainy seasons. The mosquitoes collected were: biting outdoors on donkeys (CD), biting outdoors on men (CM-OUT), biting indoors on men (CM-IN). resting indoors after blood meal (IR) and blood fed in exit trap (ET). Consistently significant differences were observed between these samples in the *a* and *b* inversion systems. The arrangements $+^a$ and *bc* were more frequent in CD, CM-OUT and ET samples than in the corresponding CM-IN and/or IR samples indicating that the mosquitoes with these chromosome types have a lower propensity to bite and rest indoors. In other words, these data point to a relationship between the 2R inversion polymorphism and differential endophily and endophagy. The substantial similarity of the chromosomal constitution of CD and CM-OUT samples indicates that differences in zoophily compared with anthropophily are not involved.

The 2R inversion polymorphism seems typically flexible (in the sense defined by Dobzhansky) as the alternative arrangements show wide seasonal and geographical changes in frequency with fairly obvious correlations with climatic and ecological conditions (Coluzzi *et al.*, unpublished). Thus those 2R arrangements which are more frequent in Jirima in outdoor samples of *An. arabiensis* ($+^a$ and *bc*) increase in frequency in southern more humid areas (Guinea Savanna), while those more frequent in Jirima in indoor samples (*a* and *b*) increase in frequency in northern more arid zones (Sahel Savanna). Data so far available for *An. gambiae* are strikingly similar. These findings suggest that the differential endophily is primarily an expression of differential climatic adaptations. Assuming a close integration of physiological and ethological responses to environmental heterogeneities, genetical variants adapted to an arid climate would be more prone to bite and rest indoors as the indoor environment generally shows a higher nocturnal saturation deficit than the outdoor environment.

Relationships between behavioural variations and inversion polymorphism have been demonstrated in the laboratory in *Drosophila* (see the review by Parsons[11]) and in *Anopheles*[12–15]. Our demonstration of such relationships in nature emphasises their evolutionary importance as well as their practical significance when dealing with a vector species. The practical implications are obvious when it is considered that a differential endophily and endophagy such as that recorded in *An. arabiensis*

Table 1 Frequencies (%) of 2R chromosome arrangements in samples of *Anopheles arabiensis* females obtained in the village of Jirima (Garki district, Kano State) during the 1973–75 rainy seasons

Collection method					Chromosome 2R					
	N	$+^a$	a	$+^b$	b	bc	$+^d$	d	be	bf
5 collections from 24.8 to 12.9, 1973										
CD	858	42.31	56.06	8.51	46.15	30.42	69.93	15.15	13.29	1.63
IR	246	30.49	68.70	9.76	54.88	21.95	67.48	19.11	12.60	0.81
χ_1^2 =		10.672†	12.096‡	0.232	5.485*	6.315*	0.431	1.936	0.030	0.416
4 collections from 31.7 to 9.8, 1974										
CD	722	48.89	48.61	6.37	49.31	31.72	75.35	12.05	10.11	2.49
IR	422	33.65	63.27	9.48	51.42	24.88	72.04	13.74	11.14	3.08
χ_1^2 =		24.591‡	22.443‡	3.266	0.395	5.694*	1.352	0.546	0.200	0.161
7 collections from 31.7 to 16.8, 1974										
ET	186	45.16	52.15	4.30	49.46	32.26	72.04	13.98	11.29	2.69
IR	772	33.81	62.05	8.16	50.78	24.22	68.91	14.25	12.69	4.15
χ_1^2 =		7.898†	5.717*	2.716	0.058	4.646*	0.554	0.0005	0.158	0.509
4 collections from 1.9 to 9.9, 1975										
CD	2262	40.19	56.45	5.97	46.86	30.59	70.51	12.91	13.22	3.36
CM OUT	1628	42.32	53.93	5.84	47.79	30.65	71.38	12.90	11.98	3.75
CM IN	780	34.23	64.23	6.92	52.56	25.26	71.79	12.95	13.72	1.54
IR	1132	31.63	65.28	8.13	51.06	23.94	69.61	13.52	13.78	3.09
χ_3^2 =		41.347‡	49.903‡	7.415	10.788*	24.063‡	1.490	0.294	2.546	8.814*

Sampling procedures used were: (1) Collection on donkeys outdoors (CD): mosquitoes were captured on 10–14 animals scattered in the village and exposed all the night. (2) Collection on men outdoors (CM-OUT): mosquitoes were captured on eight individuals from various parts of the village who had been exposed all the night. (3) Exit trap samples (ET): collections were made at 0600 h from 15 exit traps left on doors all the night. (4) Collection on men indoors (CM-IN): mosquitoes were captured on eight individuals exposed in closed huts (four per hut) all the night. (5) Indoor resting samples (IR): early morning (0600–0800 h) collections were made in 8–15 human habitations (huts) by pyrethrum spray capture or hand capture. The mosquitoes obtained from animal and human baits (mostly partly fed or unfed) were provided with a full blood meal on donkey during the same night. Blood fed specimens from all samples were kept in 200 ml paper cups (no more than 30 per cup) in favourable climatic conditions until 1400–1600 h and then processed for chromosomal examination either directly or after preservation in Carnoy fixative. No significant mortality was recorded during the maintenance period and more than 90% of the chromosome preparations were generally favourable for inversion reading.

*, †, ‡ Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

in the Garki district is expected to produce variations in vectorial efficiency and in the response to house spraying with insecticides. Some of the available data suggest that inversion polymorphism constitutes the basic genetical variability from which behavioural resistance to the insecticide may develop. Entomological investigations carried out by the WHO in the Garki district revealed important heterogeneities between *An. gambiae s.l.* from various villages, consistent with a positive association between exophily and residual density after insecticide (propoxur) spraying[16]. Such positive association clearly extends to the prespraying frequency of the 2R chromosome arrangements favouring exophily and exophagy in both *An. arabiensis* and *An. gambiae* (unpublished data).

With respect to evolutionary implications the non-random distribution of carriers of different arrangements implies some kind of ecotypic divergence among them, as suggested by Mayr[17] and supported by the work of Dobzhansky *et al.* in *Drosophila pseudoobscura*[18]. Thus the maintenance of inversion polymorphism could be based, at least in part, on annidation, in the sense defined by Ludwig[19]. A further consequence of non-random distribution of carriers of different arrangements might be their differential probability of shift to new or marginal ecological niches. This would mean a differential probability of forming isolates in which genetic reorganisation can be initiated, thus indicating the possibility of a causal involvement of inversions in speciation[20–22].

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Control of plant respiration through non-photosynthetic light action

THE effects of photosynthetically active light on carbon loss by plants are attracting much attention[1,2], but light action through mechanisms other than photosynthesis, though demonstrated as early as 1954 (ref. 3), is usually ignored in discussions of metabolic inefficiencies. Nevertheless, dim red light acting through phytochrome can clearly regulate the time course of CO_2 output in axenic cultures of *Lemna perpusilla*. The precise pattern of output under a given light schedule depends on the nitrogen source supplied; on nitrate or ammonium, the main daily peak seems to be timed in the same manner as some aspects of the photoperiodic control of flowering[4–6]. Essentially parallel results on O_2 uptake are reported here, indicating that the phenomena in question are respiratory in a reasonably strict sense.

Recording small changes in O_2 against the high background in air is essentially impossible over periods of days with methods currently available. Hence the intermittent-flow principle developed by Bruce and Street[7] for the detection of insects in stored grain was used. The change in gas concentration developed between periods of flow moves to the analyser as a 'bolus' when flow is resumed, producing a signal greater than that attainable otherwise. Comparing data so obtained with the earlier CO_2 results might be misleading, however, as intermittent flow might modify the patterns in some way. Thus all channels were also monitored with a CO_2 analyser. The first apparatus constructed had one sample channel and two blanks (without plants), and the second, two samples and three blanks. Most of the results considered are from the second version.

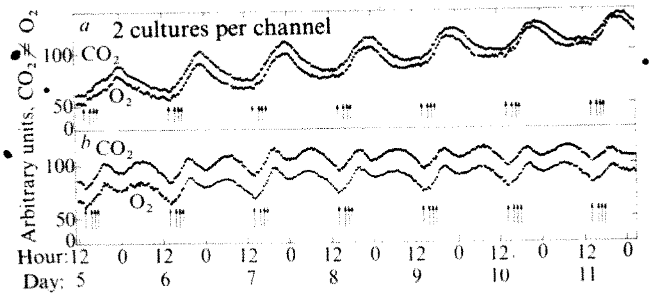

Fig. 1 Rates of O_2 uptake and CO_2 output by cultures on nitrate or aspartate as sole N sources and entrained to 4-h skeleton main light periods. Points are at 0.5-h intervals, each being assigned to the hour or half-hour immediately preceding its occurrence. The clone used was originally obtained from Dr Elias Landolt as *Lemna perpusilla* 6746, but it has also been described as *L. paucicostata*[23]. Stocks were maintained under continuous cool white fluorescent light (about 3,200 lx) at 25–27 °C, on a complete mineral medium supplemented with sucrose, yeast extract, tryptone and EDTA (refs 5, 6). Experimental cultures established by transferring batches of fronds from stock cultures to the media being tested were then capped for connection to the air streams, all substantially as in ref. 5 except that 125-ml Erlenmeyer flasks with 50 ml of medium were used instead of bottles. Nitrogen-free Z medium supplemented with either aspartic acid (*a*) or KNO_3 (*b*) at 15 mM was used, with the aspartate medium sterilised by filtration rather than autoclaving. As before, brief exposures to dim red light (cool white fluorescent screened with Rohm and Haas red 2444 Plexiglas to yield 10–35 $\mu W\ cm^{-2}$ at plant level) were used to simulate main light periods. In the results above, 15-min exposures starting at 1400, 1545, 1645 and 1745 h daily, local time (arrows), represent a 4-h main light period; exposures at 1400, 1715, 2030 and 2345 h would simulate a 10-h main light period[6]. Constant temperature in the experiment shown was about 27 °C. Cultures contained 0.2–1.4 g fresh weight of plant during the experiment, and were held in conditions being tested for 5 d before connection to the intermittent-flow gas analysis system. Each blank and sample channel received, in succession, 4 min of air flow at 50 ml min^{-1} followed by 26 min without flow. The recorded analyser outputs obtained show the samples as spikes separated by the blank values. The sample-blank differences plotted above thus represent the rates of change in O_2 or CO_2 in relative units. A cold trap (−18 °C) was interposed before the analysers to prevent water vapour from reaching them. Oxygen was determined by magnetic susceptibility using a Beckman G-2 oxygen analyser with a reversed range, nominally 21.0–20.5% (5,000 p.p.m. full scale). The infrared CO_2 analyser, a LIRA Model 303 (Mine Safety Appliances Co.) also had a full-scale range of 5,000 p.p.m. Given the intermittent-flow mode, the actual relationship between the arbitrary O_2 and CO_2 units plotted is uncertain, and will of course vary depending on the relative drifts of the two analysers. Rough calculations suggest that culture size would have to be increased 60- to 100-fold in order to obtain comparably useful data from, for example the automatic recording respirometer described by Dilley *et al*[24]: a detailed description of the present system and an assessment of its quantitative aspects will be presented elsewhere (Hillman and Tempel, in preparation). The timing of each peak can be estimated by inspection, or objectively, by summing the times of the eight or more highest values during the period in question and dividing by the total number of values, in accord with the following rules. The time of the highest value, or the times of the several highest (values of rank α) are taken first. To these are added the times of all values one unit less (rank β) and then of all of ranks γ and lower, as necessary, until at least eight values have been summed; but if one value of any given rank is included, then all others of that rank not separated from it by two or more unused lower values are also included. As an example, the peak time for the O_2 values on day 10 on nitrate, above, combines the values at 0600, 0630 and 0730 h (rank α), those at 0530, 0700 and 0800 h (rank β) and those at 0400, 0430, 0500, 0830 and 0900 h (rank γ) for a total of 11 values giving a peak time of 6.50 h after midnight, which is 16.5 h after the start of the light period, or 12.5 h after the start of the dark period.

Figure 1 summarises a typical experiment under 4-h skeleton light periods on aspartate and nitrate media. The courses of O_2 uptake and CO_2 output are essentially parallel throughout, while the patterns on the two media differ. On aspartate, there is one peak per day and on nitrate, two; earlier work with CO_2 output led to the designation of the second daily peak on nitrate as the main peak.

The time of each main peak was estimated in both the experiments shown in Fig. 1 and another performed the same way. The means and standard errors calculated from the combined experiments place the peaks in O_2 uptake on aspartate and nitrate at 7.9±0.32 and 16.1±0.18 h, respectively, after the start of each light period. The corresponding peaks in CO_2 output occur at 8.1±0.35 and 16.6±0.18 h, times not significantly different from those for O_2. All these values, as well as the patterns themselves, are essentially those obtained earlier for CO_2 output monitored by continuous flow[5,6].

In the CO_2 output data referred to, changing the length of the main light period shifts the main peak on nitrate medium. The line relating the time during the dark period at which that peak occurs to the length of the light period has a slope of −0.425±0.049 for skeleton main light periods from 4–10 h long[6]. That is, lengthening the light period by 1 h causes the peak to occur slightly less than 0.5 h earlier, as measured from the start of the dark period, which is a delay of slightly more than 0.5 h from the start of the light period. To determine whether the same relationship holds for O_2 uptake, time-course data were obtained on nitrate medium under schedules with 4- and 10-h skeleton light periods in the same experiment. For the 6 d recorded, the mean time of the main peaks under the 4-h skeleton was 16.4±0.08 h after the start of the light period, and under the 10-h skeleton, 19.5±0.18 h after the start of the light period. Subtracting the light-period lengths, this means that the 4- and 10-h main light periods place the peaks at hours 12.4 and 9.5, respectively, of the corresponding dark periods. The slope calculated from the 12 values of this experiment is −0.484±0.032. A similar calculation using values from two successive experiments carried out on the single channel version of the apparatus gave an estimated slope of −0.440±0.075. Neither of these slopes differ significantly from those derived from earlier data on CO_2 output and on flowering[6], but, like those, they do differ significantly ($P = 0.01$) from both 0 and −1.

The light effects reported earlier are probably exerted entirely through phytochrome[4,5]. Perhaps the most striking single phytochrome phenomenon is that of the 'red, far-red switch'. That is, on a nitrate medium, under a basic schedule of one 0.25-h light exposure every 24 h, cultures previously entrained to far-red respond to red by a near-doubling of CO_2 output in 6 h. Conversely, cultures previously entrained to red respond to far-red with a sharp drop in CO_2 output, even though the steady-state pattern elicited by daily far-red would give a peak shortly after the light exposure[5]. The experiment presented in Fig. 2, which may be compared to Fig. 4 in ref. 5, indicates that precisely the same effects are observed in O_2 uptake.

The fact that O_2 uptake by *L. perpusilla* is essentially parallel to CO_2 output in all conditions tested means that the use of this system to investigate photoperiodic timing[5,6] must proceed through an analysis of respiratory pathways rather than nonrespiratory decarboxylations. Beyond this rather specialised point, however, these data also lead to the conclusion that a substantial proportion of the respiration of at least one higher plant may be affected by aspects of the light regime unrelated to photosynthesis.

The applicability of this conclusion to plants in general is suggested by several considerations. Leopold and Guernsey[3] observed short-term phytochrome effects on O_2 uptake in tissues of several species. Few similar studies (for example refs 8–11) have appeared since, but there is abundant, if somewhat contradictory, recent evidence for phytochrome-mediated changes in the levels of various respiratory intermediates[12–15]. The nightly pattern of CO_2 output—presumably respiratory—by *Chenopodium polyspermum* changes within a few days of change in the length of the light period[16]. In addition, phytochrome clearly mediates light effects on rhythms in plant respiration, respiratory enzymes and intermediates[17]. Miyata and Yamamoto[18] suggested that rhythms in *Lemna gibba* respiration involve variations in the activity of the pentose phosphate pathway, while in *Chenopodium rubrum*, Deitzer *et al.*[19] found distinct rhythms in enzymes of that pathway on the one hand and of the tricarboxylic acid cycle on the other. Thus, modifications of the relative activities of these two metabolic systems might underly the present results.

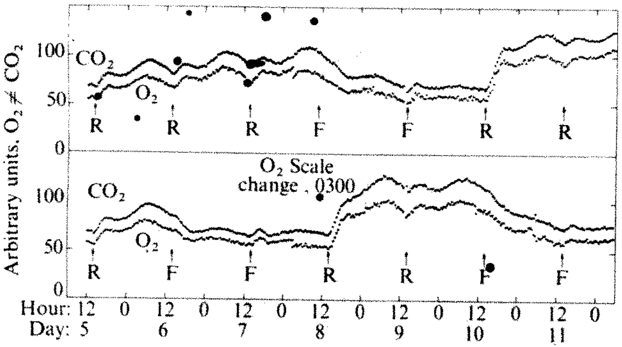

Fig. 2 Rates of O_2 uptake and CO_2 output by cultures on nitrate as sole N source and entrained originally to 0.25 h red light every 24 h, then given red (R) or far-red (F) as indicated. Light sources as in ref. 5. Other details as in Fig. 1. Gaps in the data represent recorder malfunction.

The fraction of total respiration under the control studied here has been estimated[5,6] at from 30% for daily entrainment patterns (Fig. 1) to 50% in a 'red, far-red switch' (Fig. 2). These may be minimal estimates. Unpublished data indicate that respiration in most of the cultures used is soon limited by crowding, which probably explains the apparent decrease in the proportion of control with time (Fig. 1 of ref. 5). While the parallelism between O_2 uptake and CO_2 output is extremely close in these data, it may not be perfect. The ratio of the arbitrary values, or apparent respiratory quotient, changes slightly in response to experimental variables. Further work will be required to establish that these observations do not result merely from differing gas solubilities or other nonbiological factors.

Finally, although the term photorespiration has become virtually synonymous with effects of bright light on the consumption of current or very recent photosynthate, a comprehensive view of metabolic regulation should no longer ignore the fact that overall respiratory rate can be massively, and on occasion rapidly, modified through other light actions. Since these actions may be exerted at times remote from those of actual illumination, and at low energies, the retention of quotation marks in the phrase (and concept) of 'dark' respiration[2] seems particularly wise. The fact that the respiratory rhythm in *Lemna gibba* seems to be correlated with changes in azide sensitivity[18] is of particular interest since inhibitor-resistant electron transport may be wasteful with respect to phosphorylation[20]. Thus, in the context of current concern with metabolic efficiency and plant productivity[1,2], this work directs renewed attention to light action on phytochrome and to its timing—the photoperiodic aspects of the daily light regime. And although the effects of blue light on *Lemna* respiration are probably mediated entirely by phytochrome[4], the situation in general could prove to be even more complex, in view of recent evidence for physiological photoreception by flavin-cytochrome b couples in both fungi[21] and higher plants[22].

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Viral contamination of bovine foetal serum and cell cultures

THE presence of adventitious viruses in cell cultures is well recognised[1], and when the cultures are of primate origin there are serious hazards for the production of human viral vaccines[2]. This is one reason for the increasing use of bovine cell cultures. These cultures, however, are not free from viral contamination[3]. We found that calf kidney (CK) and calf testis (CT) cells were often infected by non-cytopathic mucosal disease virus (MDV): the cells seemed morphologically healthy but nearly all showed fluorescence with MDV antiserum and rabbit-anti-bovine conjugate. We report here that both the cells and foetal calf serum, an essential growth factor of cell culture medium, are sources of the virus.

MDV (also known as bovine viral diarrhoea virus), a member of the family Togaviridae, is antigenically related to hog cholera virus[4] and morphologically similar to rubella virus[5]. The role of FCS, as a source of contamination by MDV, was examined by growing CT cells in medium supplemented with either unheated or heated FCS. Heated FCS had been held at 56 °C for 30 min because these condition inactivated MDV. The incidence of infection of each cell batch grown in unheated FCS was compared with the incidence of infection of the same cell batch grown in heated FCS (Table 1). Fourteen batches of CT cells grown in five batches of FCS were examined. All of the 14 batches of cells grown in the presence of unheated FCS became infected by MDV. In contrast, when grown in the presence of heated FCS, only one of the 14 batches became infected by the virus.

Each batch of CT cells was screened for infection by MDV at several different passage levels (Table 2). Viral infection was not usually detected until passage 6 when 70% of cell batches grown in unheated FCS showed MDV fluorescence. Only 25% of cell batches showed evidence of MDV infection at passage 3.

Table 1 Batches of bovine cell cultures showing MDV fluorescence and grown in the presence of unheated or heated FCS

Cell	FCS	No. positive	No. tested
CT	Unheated	14	14
	Heated	1	14
CK	Heated	10	63
MDBK*	Unheated	1	1‡
BT22†	Unheated	1	1‡

The indirect fluorescent antibody test was carried out using antiserum raised in a gnotobiotic calf against the NADL (National Animal Diseases Laboratory) strain of MDV, and a rabbit-anti-bovine serum conjugated with fluorescein (Nordic Immunology). Pre-inoculation serum was used as a control of fluorescence. CT and CK cell cultures were derived from 1–15 d-old calves, freshly slaughtered each week; trypsinised tissues were seeded into 20-oz bottles and cultured in Eagle's basal salts medium supplemented with 7.5% commercial FCS. Each batch of CT cells was subcultured for up to 15 passages, and CK cell cultures were subcultured until the secondary passage.

*Madin Darby bovine kidney.

†Bovine turbinate.

‡Continuous cell lines; therefore only one 'batch' could be examined.

The other possible source of non-cytopathic MDV, the cells themselves, was examined by fluorescent antibody staining of CK cells which were grown in the presence of heated FCS and subcultured only once. Ten of 63 cell batches examined showed MDV fluorescence. Two continuous cell lines, MDBK and BT22, were also found to be infected by non-cytopathic MDV (Table 1).

Specificity of the fluorescent antibody test was confirmed by neutralisation tests. The agent isolated from CT cells was able to infect uncontaminated CK cells. But when the agent was first treated with antisera to MDV (either a Weybridge reference serum or an antiserum raised at Compton by hyperimmunisation of a gnotobiotic calf with the NADL–MDV strain), CK cells did not become infected. Thus antisera to MDV neutralised the agent isolated from CT cells; no neutralisation occurred with pre-immune control serum.

The results of fluorescent antibody staining were also supported by tests for interference, which were carried out by a plaque technique. A standard inoculum of cytopathic MDV was titrated on Petri dishes of CT cells, which fluorescent antibody staining had shown to be infected by non-cytopathic MDV. The number of plaques produced by the cytopathic virus was compared with the number produced by the same viral inoculum titrated on known, uncontaminated CT cells (Table 3). No more than a twofold reduction in plaque number on the contaminated cells was found.

Table 2 CT cell cultures showing MDV fluorescence at various passage levels

FCS*	Results of FA†	Cell passage no. 1	3	6	9	12	15
Normal	No. positive	0	3	10	8	7	6
	No. tested	6	12	14	12	8	6
Heated	No. positive	1	0	1	1	1	0
	No. tested	11	12	14	12	8	6

*Half of each batch of CT cells was grown on medium supplemented with unheated FCS and half on medium supplemented with heated FCS.

†Indirect fluorescent antibody staining as in Table 1.

The conventional interference test, used for screening FCS and bovine cells, relies on detection of a visible inhibition of virus-induced cytopathic effect. This method is obviously not as sensitive as a quantitative plaque technique and probably would not detect the relatively low level of interference as noted in Table 3.

The results indicate two different sources of contamination by non-cytopathic MDV. First, MDV may be found in commercially produced FCS because heat treatment of serum reduces the incidence of infection of CT cells from 100% to 10%. Second, MDV may be indigenous to bovine cells; approximately 20% of batches of CK cells grown in heated FCS for only two passages, and on serum shown to be free of MDV by culture of CT cells, are infected by MDV. CT cells grown in normal FCS have to be subcultured several times before showing evidence of MDV infection, and so the viral inoculum in FCS is low.

Table 3 Comparison of number of plaques produced by cytopathic MDV on uninfected CT cells with number of plaques produced on CT cells infected by non-cytopathic MDV

Cell batch/ passage no.	Mean no. plaques per plate ± s.e. Uninfected	Infected	Student's t test
60/9	48±8	24±7	5.131
60/13	21±7	15±2	1.774*
63/6†	54±12	26±3	4.704
	29±8	17±4	2.998
	30−5	18−5	3.485

Standard inocula of cytopathic MDV (NADL) were titrated on Petri dishes of CT cells either contaminated or uncontaminated by non-cytopathic MDV. Mean numbers of plaques produced on five replicates of contaminated CT cells were compared with mean numbers of plaques produced on the same cells but which were uncontaminated. Student's t test, $P<0.05$, 4d.f.

*All comparisons except this one showed significant differences.

†Three different inocula of cytopathic MDV.

In the above experiments, CT cell cultures were exposed to five different batches of FCS obtained from one commercial supplier: all batches contained MDV. These results indicate a much higher incidence of contamination than reported previously[6,7], and illustrate the inadequacy of commercial screening methods. This inadequacy may be explained by the relative insensitivity of the interference test, often the only method used to detect non-cytopathic MDV, and by the fact that the amount of virus in FCS is probably small.

The presence of MDV in FCS is explained by the ability of the virus to infect the bovine foetus *in utero*[8]. A main supplier of FCS is Australia where infection by MDV is widespread: in 1964 it was shown that approximately 60% (374/625) serum samples taken from cattle throughout the country had neutralising antibodies to MDV[9]. The incidence of foetal infection is unknown, but because each batch of FCS represents a pool of serum taken from about 500 foetuses (for an average batch of 170 l), contamination of a batch requires only a 0.2% incidence of foetal infection by MDV.

Infection of bovine cell cultures by non-cytopathic MDV has particular significance for the production of viral vaccines. Tamoglia[10] tested live infectious bovine rhinotracheitis vaccines from all licensed producers in the United States and found that 8% were contaminated by MDV. Vaccination with such vaccines may give rise to abortions since transplacental infection by MDV can cause death and malformations of the foetus[11].

The importance of contamination by MDV with regard to human viral vaccines is unknown, but measles virus vaccine[12] and a potential respiratory syncytial virus vaccine[13] are produced on bovine kidney cells grown in the presence of unheated commercial FCS. Contamination of viral stocks also has important implications for experimental virology. For example, in a trial designed to assess the clinical responses of calves to infection by respiratory syncytial (RS) virus[14], the RS viral inoculum, grown in CK cells, was found to contain MDV. If MDV had not been detected, the clinical reactions of inoculated animals would have been attributed solely to RS virus.

Regular screening of bovine cell cultures is essential if viral stocks are to be kept free from MDV. We suggest that the fluorescent antibody test, using a monospecific antiserum, is the most reliable method available for detecting non-cytopathic MDV in cell cultures. Moreover, if FCS is heated before use, the incidence of contamination of cell cultures by MDV will be greatly reduced.

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Neonatally tolerant mice fail to react against virus-infected tolerated cells

IN mice, immune effector functions mediated by thymus-derived lymphocytes (T cells) are restricted by the major histocompatibility (*H–2*) gene complex (see ref. 1 for review). Thus, virus-stimulated cytotoxic T cells are specific for both viral antigen(s) and self-cell-surface structures coded in the *K* or *D* region of *H–2* (refs 2, 3). Two general hypotheses have been proposed to explain *H–2* restriction[2–5]. First, T cells possess two independently, clonally expressed, but linked receptors, one for the foreign antigenic determinant and one for the relevant self-marker (dual receptor model); second, T cells have a single clonally distributed receptor for a complex of foreign antigen plus self or for virally altered self-markers (altered self model). To date no conclusive proof exists for one hypothesis or the other. The altered self model is the simpler concept of the two; on the other hand, the fact that cytolytic interactions against minor histocompatiblity antigens are *H–2* restricted and that the idiotypic determinants of T cell-receptors are identical with those of immunoglobulins of the same specificity[6–8] are probably more compatible with a dual receptor.

In both models, but particularly in the dual receptor model, defining the relationship between tolerance against alloantigens and the capacity to recognise the same alloantigens as self or alternatively as altered self seems to be crucial for understanding of the mechanisms of T cell recognition. Experiments with $H\text{–}2^k/H\text{–}2^d$ radiation chimaeras, that were reconstituted with one parent's haemopoietic stem cells revealed that separate groups of T cells were specific for virus plus $H\text{–}2^k$ or virus plus $H\text{–}2^d$ (refs 9–11). The absence of alloreactivity (presence of tolerance) appeared to parallel presence of self-recognition (as proposed in the dual receptor model), or alternatively the presence of recognition of altered self. The present experiments were designed to evaluate whether tolerance to alloantigens and reactivity to tolerated self plus virus parallel each other when tolerance was maintained with minimal chimerism as in the classical Billingham–Brent–Medawar[12] neonatally tolerant mouse model. The results revealed no such correlation.

Neonatal A.AL ($H\text{–}2K^k$) mice were injected into the orbital vein with spleen and bone marrow cells of (A.AL × A.TL)F$_1$ ($H\text{–}2K^k/H\text{–}2K^s$) origin. (W. Streilein and J. Klein, in preparation). Eight weeks later, survivors were grafted with A.TL skin. Mice that did not reject the skin grafts were considered as tolerant ($H\text{–}2K^k$ tolerant to $H\text{–}2K^s$). Tolerant and conventional A.TL ($H\text{–}2K^s$), SJL ($H\text{–}2K^s$) and B10.BR ($H\text{–}2K^k$) mice were then infected with vaccinia virus, and their spleen cells were assayed for virus-specific cytotoxic activity on vaccinia virus infected and uninfected syngeneic or allogeneic target cells 6 d later (Table 1). The degree of chimaerism of the tolerant mice was determined by lysing of their lymphocytes in the cytotoxic test with antisera against *H–2K* haplotypes. Chimerism in the tolerant mice was below 5% since specific anti $H\text{–}2K^k$ (K-603) antiserum + C′ killed > 95% of lymphocytes from conventional A.TL mice, lysed less than 5% cells from tolerant mice[13,14].

Although vaccinia virus immune spleen cells from tolerant mice lysed infected $H\text{–}2K^k$ L cells to a high degree they failed to lyse any of the three infected $H\text{–}2K^s$ targets. The $H\text{–}2K^s$ targets were lysed very efficiently by A.TL or SJL immune lymphocytes. Therefore, the efficiency with which virus immune spleen cells from allogeneic or tolerant mice lysed the $H\text{–}2K^s$ targets must be at least 50 times lower than that for immune cells from A.TL or SJL.

This result could suggest the presence of some suppressor mechanism in the tolerant mice's immune spleen cells that prevent expression of virus-specific cytotoxic activity against the infected targets of the tolerated *H–2* type. Yet the cytolytic activity of virus specific T cells specific for K^s was not diminished significantly when they were mixed with excess numbers of cells from mice tolerant to K^s; any effects observed were comparable to interference caused by unrelated K^d spleen cells from B10.D2 mice that were not tolerant to K^s (Table 2). Thus suppression seems to be unlikely at least at the level of the effector cell. This experiment, however, cannot exclude the possibility that suppressive mechanisms act during induction of the cytotoxic lymphocytes.

The difference of the results obtained in mice that were rendered tolerant neonatally and the F1 radiation chimeras may reflect different mechanisms of tolerance induction and/or maintenance. Since these mechanisms are poorly understood this argument remains open. A possible difference may be presence or absence of suppression. Alternatively, the data may suggest that the generation of self plus virus-specific cytolytic T cells is, besides being virus-dose dependent, also dependent on the dose of the relevant self-markers. In P ($H\text{–}2^k$)→F$_1$ ($H\text{–}2^k/H\text{–}2^d$) radiation chimeras, when the syngeneic and the tolerated allogeneic haplotypes are both expressed in great amounts, virus-specific cytolytic activity is generated against virus in association with both

Table 1 Vaccinia virus specific cytotoxic T cell activity of neonatally tolerant mice

Immune spleen cells (mouse strain, H–$2K$ allele)	Spleen to target cell ratio	% ^{51}Cr release from target cells* L929 (H–$2K^k$)		SJL fibroblasts (H–$2K^s$)		SJL macrophages (H–$2K^s$)		A.SW macrophages (H–$2K^s$)	
		Vaccinia	Normal	Vaccinia	Normal	Vaccinia	Normal	Vaccinia	Normal
A.AL tolerant to A.TL (H–$2K^k$ tolerant to H–$2K^s$)§	30:1	78.4†	22.7	20.9‡	15.3	38.0‡	40.5	39.9‡	34.7
	10:1	92.4†	24.8	21.8‡	—	39.4‡	—	40.7‡	37.0
	3:1	65.4†	20.2	21.2‡	—	39.6‡	—	42.0‡	35.2
A.TL (H–$2K^s$)	30:1	16.6	24.2	76.6†	18.1	88.0†	42.1	70.4†	35.2
	10:1	17.9	23.2	48.1†	—	73.1†	—	57.0†	36.2
	3:1	15.9	—	37.5†	—	56.1†	—	51.9†	—
SJL (H–$2K^s$)	30:1	16.9	—	—	—	83.2	41.0	—	—
	10:1	19.9	—	—	—	85.0	—	—	—
	3:1	—	—	—	—	72.4	—	—	—
B10.BR (H–$2K^k$)	30:1	74.2†	20.1	19.8	—	43.1	41.7	43.3	—
	10:1	66.1†	—	18.5	—	42.6	—	—	—
	3:1	45.7†	—	—	—	41.7	—	—	—
Normal A.TL (H–$2K^s$)	30:1	12.2	—	—	—	40.2	40.3	41.9	32.2
	10:1	13.6	—	—	—	42.7	—	—	34.2
Normal B10.BR (H–$2K^k$)	30:1	14.2	20.7	—	—	—	—	—	—
	10:1	—	—	—	—	—	—	—	—
Medium		12.3	19.5	14.0	18.2	40.3	41.0	42.0	33.0

Mice were infected with about 10^7 plaque forming units (PFU) of vaccinia virus (WR strain). The mice were killed 6 d later and spleen cells assayed for cytotoxicity at the spleen cell to target cell ratios indicated. Since only the constitution of the K region of the H–2 complex is relevant to this experiment, the constitution of the other regions is not shown. Where no values are shown the values were not determined.

*L929 continuous line fibroblast cells, SJL secondary fibroblasts and SJL or A.SW peritoneal macrophages were labeled with ^{51}Cr, washed and infected with vaccinia virus at a multiplicity of about three for 3 h at 37 °C. The % of ^{51}Cr released was determined after 6 h incubation at 37 °C. The standard errors did not exceed 2.2 for all targets tested.

†Significantly greater than controls ($P < 0.01$).

‡Not significantly different from normal cell controls or medium.

§The H–2 type of lymphocytes was determined by a microcytotoxicity test[13,14]

	% specific lysis anti–K^s+C′ (K–119)	anti–K^k+C′ (K–603)	C′ alone
A.TL	>95	>5	>5
Tolerant	<5	>95	<5

The antisera were K–603 : (C3H.OH × 129/J)F$_1$ anti–C3H/HeJ, and K–119 : (A × C57BL/10Sn)F$_1$ anti–B10.S.

Table 2 Absence of demonstrable blocking or suppressing activity on virus-specific cytotoxicity in spleen cells from neonatally tolerant mice

Immune spleen cells (mouse strain, H–$2K$, D allele)	Spleen to target cell ratio	Numbers of blocking cells added	% ^{51}Cr release from vaccinia virus infected target cells: SJL macrophages (H–$2K^sD^s$) Admixed blocking immune spleen cells None	Tolerant	B10.D2	L929 (H–$2K^kD^k$)	J774 (H–$2K^dD^d$)
A.SW (H–$2K^sD^s$)	30:1		78.9	—	—	25.3	30.0
	10:1		84.4	—	—	21.2	30.5
	3:1		65.7	—	—	18.7	—
B10.D2 (H–$2K^dD^d$)	30:1		52.0	—	—	24.2	92.7
	10:1		50.7†	—	—	22.0	80.4
	3:1		—	—	—	20.7	56.2
A.AL tolerant to A.TL (H–$2K^kD^d$ tolerant to H–$2K^s$)	30:1		56.1†	—	—	90.7‡	73.6‡
	10:1		58.1†	—	—	87.4‡	57.3‡
	3:1		55.1†	—	—	54.1‡	39.5
A.TL (H–$2K^sD^d$)	30:1		79.8‡	—	—	22.0	81.2
	10:1	1×§	89.1‡	81.6*	89.4	22.3	57.1
		2×	—	84.7*	76.7	23.7	39.0
	3:1	1×	70.1‡	70.9*	69.6		
		2×	—	67.8*	68.9		
		9×	—	61.1*	60.5		
Normal A.TL			55.6	—	—	23.0	34.7
Medium			54.2	—	—	25.1	33.0

Mice were infected and cytotoxicity tests performed as for Table 1. S.e.m. were below 2.5 except for the blocking data where they were below 3.2.

*None of these values was significantly smaller than those obtained with B10.D2 as blocking cells.

†Not significantly greater than controls.

‡Significantly greater than controls, $P < 0.01$.

§Vaccinia immune spleen cells from the B10.D2 or tolerant mice tested were added to the A.TL immune spleen cells in equal amounts (1×) or 2, 3 and 9 times excess amounts.

The H–2 type of the lymphocytes was determined by microcytotoxicity test[13,14].

	% specific lysis anti–K^s+C′ (K–119)	anti–K^k+C′ (K–603)	C′ alone
A	<5	>95	<5
A.TL	>95	<5	<5
Tolerant	<5	>95	<5

haplotypes. In the neonatally tolerant mice studied here, the chimaerism is minimal, sufficient to maintain tolerance, but not to an extent where during infection appreciable amounts of cells would express virus plus alloantigen to trigger measurable quantities of cytotoxicity against these specificities.

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Reassembled HVJ (Sendai virus) envelopes containing non-toxic mutant proteins of diphtheria toxin show toxicity to mouse L cell

PREVIOUS studies from this laboratory have described the preparation, from HVJ virions solubilised with a non-ionic detergent, of a reassembled envelope fraction free of nucleocapsids. Such reconstituted envelopes still retain neuraminidase, haemagglutinating, cell fusion and haemolytic activities[1-3]. Since intact virions release their nucleoproteins into the cytoplasm after fusion with mammalian cells, it seemed possible that any given macromolecule contained within such a reassembled viral envelope might be introduced into susceptible mammalian cells by this means. We report here the successful application of this method to the introduction of CRM45, a non-toxic mutant protein related to diphtheria toxin[4], into mouse L cells, a strain that is normally toxin resistant. Although CRM45 is a potent NAD : elongation factor 2-ADP ribosyl (NAD : EF2-ADPR) transferase[5,6] and thus blocks protein synthesis in eukaryotic cell extracts, it is non-toxic for animal cells because it lacks a C-terminal sequence of amino acids necessary to bind to susceptible cell membranes and thus cannot reach the cell interior[7]. If a few molecules of CRM45 could be introduced into the living cell cytoplasm, they should block protein synthesis and kill the cells. This has now been accomplished by fusion of CRM45-containing reassembled HVJ envelopes with cultured mouse L cells.

HVJ virions were collected by centrifugation, resuspended in buffer containing CRM45 and divided into two portions. Non-ionic detergent Nonidet P40 (NP40) was added to one portion to solubilise the virions; it was not added to the other which served as a control. After ultracentrifugation of the solubilised virions to remove nucleocapsids and other insoluble material, the envelopes were reassembled by dialysis against phosphate buffered saline (PBS) to remove the detergent and then applied to a Sephadex G200 column to separate reassembled envelopes or native virions from free CRM45 protein. The reassembled envelope fraction was eluted from the gel with the void volume as a single sharp peak (Fig. 1). NAD : EF2-ADPR transferase activity and haemagglutinating activity were both associated with this peak. A peak of free CRM45 was found in fraction 41 and thus was well separated from the reconstituted envelopes. The CRM45-containing reassembled envelopes were collected and resolubilised with NP40. Resolubilisation resulted in a 3.2-fold increase in enzymatic activity (Table 1). These observations suggest that about 70% of the CRM45 was inside the reassembled envelopes and about 30% was tightly bound to the membrane with its enzymatically active fragment A moiety[8,9] exposed to the outside. About 2% of the total added CRM45 was recovered with the envelope fraction. The control HVJ virions, suspended in CRM45 at the same concentration, were also filtered through Sephadex G200. The native virion fraction emerged with the void volume as expected, but contained very little ADP ribosylating activity. There was no further increase in enzymatic

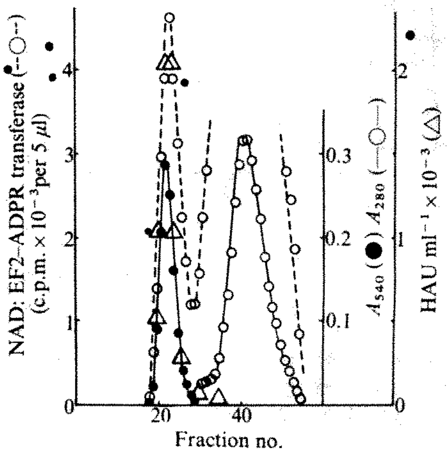

Fig. 1 Separation of reassembled CRM45-containing envelopes from free CRM45. Purified HVJ virions (about 7×10^4 HAU) were collected and resuspended in 1 ml 0.005 M Tris buffer at *p*H 7.4 containing 5 mg ml^{-1} CRM45 and divided into two equal portions. To the first was added NP40 at a final concentration of 0.25%; the other served as a control. After 15 min at room temperature, the solubilised virions were centrifuged at 40,000 r.p.m. for 60 min to remove nucleocapsids and other insoluble material. The resulting supernate was dialysed at 4 °C in a Neflex tube against PBS with the buffer changed each day for 3 d. The reassembled dialysed envelope preparation was then chromatographed through Sephadex G200. Ten drop fractions (0.73 ml) were collected and the absorbance of each fraction was measured at 280 nm and at 540 nm. The HVJ virions to which no NP40 had been added were suspended in 0.5% CRM45 solution and dialysed against PBS and chromatographed through Sephadex G200 in the same manner. NAD:EF2-ADPR transferase activity of each fraction was assayed using 2 μl, 5 μl and 10 μl samples by a slight modification of the method of Gill and Pappenheimer[8]. Haemagglutinating activity, determined using chicken erythrocytes, was expressed as HAU ml^{-1}.

Table 1 Enzymic activity of CMR45-containing reassembled envelopes before and after treatment with NP40

Pooled fractions	A_{540}	HAU ml^{-1}	NAD:EF2-ADPR transferase before NP40 c.p.m. per 5 µl	NAD:EF2-ADPR transferase after NP40 c.p.m. per 5 µl
Reassembled envelopes containing CRM45	0.225	1,500	4,115	12,963*
Intact virions, preincubated with CRM 45	1.5	16,000	187	150

*This activity increased about twofold as did that of free CRM45 after brief controlled treatment with trypsin (see ref. 4).

Fractions 21–24 of Fig. 1, both from gel filtration of CRM45-containing reassembled envelopes and from the intact HVJ virions, were pooled. The pooled volume was 2.9 ml in each case. Both suspensions were then solubilised with NP40. Assay for NAD:EF2-ADPR transferase activity was carried out on 2 µl and 5 µl samples after threefold dilution, both before and after treatment with NP40 as described in the legend to Fig. 1.

activity after solubilisation of the virions with detergent (Table 1). Thus CRM45 neither enters the intact virions nor binds non-specifically to their surface.

The enzymatic activity per ml of CRM45-containing envelope suspension was equivalent to about 20 μg ml^{-1} of diphtheria toxin. The toxicity of the suspension was tested using mouse L cells known to be at least 10,000 times as resistant to intact toxin as HeLa cells. Table 2 shows that 70% and 92% of L cells were killed after short exposure to the CRM45-containing HVJ envelope suspension diluted so as to contain respectively only 10 and 100 haemagglutinating units (HAU) per ml. The same amount (HAU) of either reassembled virion envelopes, or of ultraviolet-killed virus, whether added alone or mixed with free CRM45, showed no toxicity at all for L cells when tested under the same conditions. It is clear that CRM45-containing reassembled HVJ envelopes are toxic for a mammalian cell line. Further experiments suggest that the method is likely to prove of general use for introducing macromolecules into the cytoplasm of living mammalian cells. We experienced no difficulty in introducing ^{125}I-labelled IgG into mammalian cell lines (L cell, Ehrlich ascites tumour cell and KB cell). The yield of IgG trapped within the reconstituted viral envelopes was about the same as for CRM45.

Table 2 Toxicity of CRM45-containing reassembled HVJ envelopes for mouse L cells

Sample	Number of surviving colonies per dish 100 HAU ml^{-1}	Number of surviving colonies per dish 10 HAU ml^{-1}
Reassembled CRM45-containing envelopes	12	44
Intact HVJ virions plus CRM45 (1 µg ml^{-1})	132	129
Reassembled empty envelopes plus CRM45 (1 µg ml^{-1})	132	129
Reassembled envelopes	142	155

Two hundred L cells suspended in 2 ml of MEM medium containing 10% calf serum were added to 30 mm plastic Petri dishes and incubated for 7.5 h at 37 °C. The cells were then washed once with 2 ml chilled buffered salt solution (BSS, see ref. 15) before 0.5 ml of the chilled sample was added. After 20 min at 4 °C they were brought to 37 °C and incubated a further 20 min. Two ml culture medium was added and incubation continued for 3 h at 37 °C. The medium was then exchanged for fresh medium and the cells were cultured for 7 d. The cells were finally fixed with methanol and stained with Giemsa. Colony counts were averages of duplicate plates. Control dishes contained 130 and 132 colonies. To prepare samples for test, the pooled fractions of reassembled HVJ envelopes of Table 1 were diluted in BSS 15 and 150 times to final concentrations of 100 and 10 HAU ml^{-1} respectively. Both ultraviolet-killed HVJ virions and their envelopes reassembled in the absence of CRM45 were diluted to 100 and 10 HAU ml^{-1}. CRM45 was added to the diluted controls at a final concentration of 1 µg ml^{-1}.

There are many reports of introduction of substances into living mammalian cells, the most frequently used techniques being the liposome[10–12] and erythrocyte ghost–HVJ methods[13,14]. As the procedures for reassembling the envelopes are mild without any drastic mechanical steps and the reassembled HVJ envelopes have specific binding sites to susceptible cells, we believe that the method we have described is also effective for introducing proteins into the cytoplasm of living mammalian cells.

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T lymphocyte responses to *Mls* locus antigens involve recognition of *H-2 I* region gene products

THE major histocompatibility complex (MHC) in the ninth linkage group of the mouse encodes antigens that stimulate strong alloreactions[1]. *In vitro* studies have focused on two such reactions, both mediated by T lymphocytes. Cytotoxic T cells can recognise products of the MHC *H-2K* and *D* regions (KD antigens), and can be distinguished by their Ly antigen phenotype from the non-cytotoxic T lymphocytes that give their strong proliferative responses in mixed leucocyte culture (ref. 2) (MLC). These latter cells respond primarily to *H-2 I* region gene products[1,2]. The products of a second genetic locus in the mouse also stimulate strong MLC responses. This *Mls* locus[3] behaves as a single autosomal dominant gene mapping in linkage group 13. It has been defined by studying MLC reactions between MHC identical strains of mice. Interestingly, it has not been possible to induce cytotoxic effector T cells against *Mls* locus antigens[4]. Recent interest in T cell responses to antigen has focused on the crucial role of MHC gene products in antigen recognition (reviewed in refs 5–8). These studies have shown that T cell responses to most conventional antigens involve recognition of both the foreign antigen and self MHC antigens. In parallel with allogeneic reactions, T cells cytotoxic to antigen-modified self cells see that antigen plus self KD molecules, while helper T cells, T cells mediating delayed type hypersensitivity and T cells proliferating *in vitro* to soluble antigens recognise the antigen in association with I region gene products. Thus,

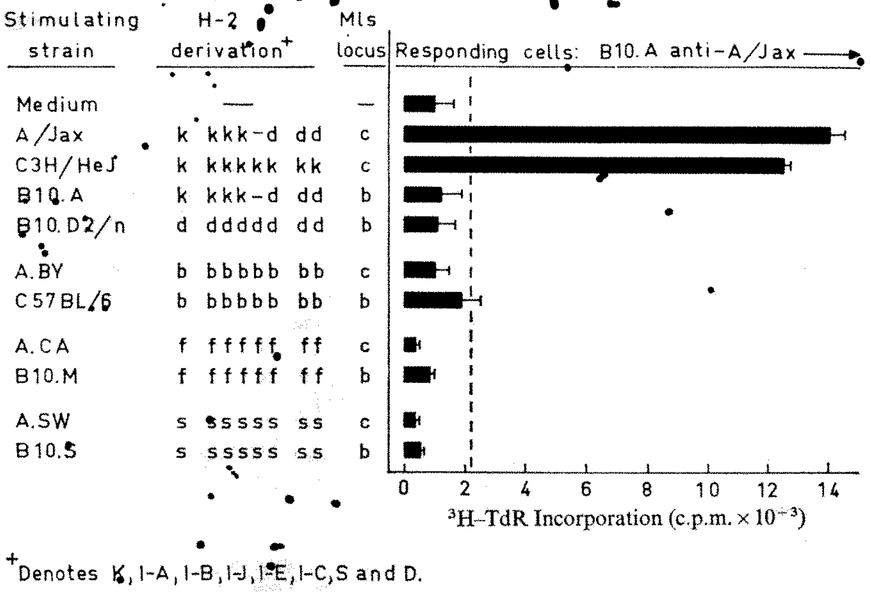

Fig. 1 Secondary stimulation of B10.A T lymphocytes primed *in vitro* with X-irradiated A/Jax spleen cells by a panel of spleen cells. The responding B10.A T cell population for primary MLC was prepared by the passage of whole spleen cell suspensions whose erythrocytes had been lysed with 0.84% NH_4Cl through Ig-anti-Ig glass bead columns, according to the protocol of Wigzell *et al.*[15]. Primary MLC were performed in tissue culture flasks (No. 3013, Falcon Plastics) and consisted of 30×10^6 responding T cells cultured with an equal number of X-irradiated (2000 R) stimulating cells in 12 ml of EHAA medium supplemented with 0.5% normal mouse serum[10,16]. The reacting T lymphocytes from the primary MLC were separated as T blast cells from the non-reacting cells using 1*g* velocity sedimentation gradients as described elsewhere[10]. 7.5×10^4 T cell blasts (> 95% blasts) were dispensed into microtitre plate wells, incubated 12 h, then restimulated with the indicated cell populations ('stimulating strain'). Secondary cultures were performed according to Peck *et al.*[10]. After 48 h of secondary MLC, the cultures were pulsed with 0.8 μCi tritiated thymidine (Radiochemical Centre, Amersham) in 20 μl for 4 h. Thymidine incorporation in harvested cells was determined by liquid scintillation counting. Data are expressed as mean c.p.m. of triplicate culture wells. Standard deviations are shown. Vertical dashed line is 2 standard deviations above the mean of incorporation in medium stimulated controls.

virtually all T cell responses involve recognition of MHC gene products, with the possible exception of responses to *Mls* locus antigens. Reactions to *Mls* locus antigens, however, are normally tested between MHC identical strains of mice, so responses to *Mls* locus antigens may also involve recognition of self MHC antigens, probably encoded in the I region[7]. Alternatively, the *Mls* locus might represent I region gene duplications translocated to another chromosome[3,9]. The present experiments were designed to test these ideas. We report that T cell responses to *Mls* locus antigens involve recognition of the products of two gene clusters. One gene cluster is clearly self *MHC* genes, probably within the I region, while the second is almost certainly the genes of the *Mls* locus. Thus, virtually all T cell responses to non-MHC antigens involve specific recognition of self-MHC antigens in association with another antigenic determinant.

We have isolated T cell blasts from primary MLC reactions between *H-2* identical, *Mls* locus differing strains, and measured their secondary *in vitro* proliferative response to fresh stimulating cells from strains sharing either MHC antigens, *Mls* locus antigens, or both with the original stimulating strain[10]. This technique facilitates study of reactions to *Mls* locus antigens in the presence of MHC antigen differences, since the selected blast cells lack reactivity to MHC antigens, at least during the time in which they are studied (48 h).

Two such experiments are presented. The first (Fig. 1) involved blast cells isolated from the reaction B10.A anti-A, and tested with a variety of strains congenic with A but differing in the MHC. It is clear that secondary reactions to *Mls* locus antigens require that the stimulating cell share *MHC* region genes with the original stimulating strain. Sharing of the rest of the genetic background is not sufficient to give a response. Since C3H/HeJ cells induced reactivation, sharing MHC genes to the left of the I-C sub-region seems to be sufficient. Sharing of *Mls* locus genes with the original stimulating strain is apparently also required for this response, but not sufficient.

A second experiment was designed to determine which portion of the MHC was involved in secondary T cell responses to *Mls* locus antigens (Fig. 2). In this instance, purified T cell blasts from the reaction of B10.BR to

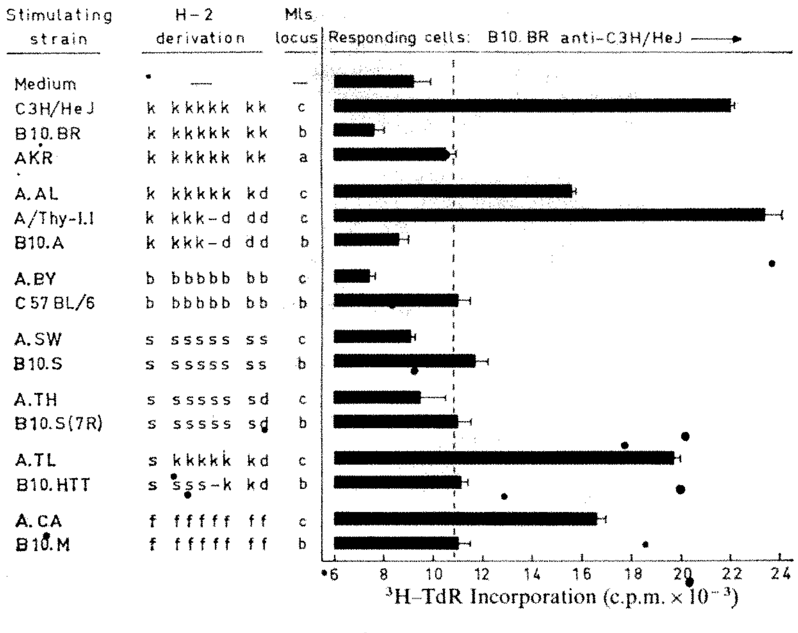

Fig. 2 *H-2* I region recognition in secondary MLC responses of B10.BR T lymphocytes primed *in vitro* with X-irradiated C3H/HeJ spleen cells towards a panel of test cells carrying various genetic differences. Primary and secondary MLC carried out as for Fig. 1.

C3H/HeJ were used. C3H/HeJ and all A congenic mice share an Mls locus antigen (Mls^C), allowing us to test the A congenic mice for secondary stimulatory activity. B10 congenic strains were used as *Mls* negative controls for any reaction to MHC differences. The results confirm the requirement for sharing MHC region genes with the original stimulating strain. Furthermore, the reactions against A.TL and A/Thy-1.1 cells demonstrate that genes mapping between K and I-C are sufficient for stimulation to occur. These experiments do not rule out the possibility of other MHC genes also being sufficient to give stimulation in this system, but both experiments demonstrate an absolute requirement for sharing of MHC genes, since the congenic mice differ only at the MHC region.

These data strongly support the notion that T cell recognition of non-MHC antigens always involves recognition of self MHC gene products. Other non-MHC cell surface antigens have been shown to behave in a similar manner. The male antigen[11] and non-H-2 transplantation antigens[12] both induce killer T cells which recognise both the antigen and KD determinants. The *Mls* locus antigens are unique, however, in being the only non-*H-2* antigens which induce a strong primary proliferative, non-cytotoxic T cell response; our data suggests they do so in association with I region gene products. Antigens with the characteristics of Mls antigens all map to a single locus, whereas there are a wide variety of non-*H-2* loci encoding antigens that give rise to cytotoxic effector cells[11,12], so this uniqueness may, in part, account for the apparently high frequency of *Mls* reactive cells.

These experiments render very unlikely the hypothesis that *Mls* locus products resemble I region gene products, since *Mls* locus antigens cannot, by themselves, induce T cell proliferation, but must be associated with an I region gene product to do so. Other differences between I region gene products and *Mls* locus antigens emerge from studies of T–B cell collaboration, where I region identity is required for effective cooperation[13] while *Mls* locus identity is not[14].

Finally, we believe that this system can be applied to the mapping of I region genes. A possible illustration of this is the finding that B10.A anti-A cells do not respond to A.CA, whereas B10.BR anti-C3H cells do respond to A.CA. This reaction pattern does not correspond to any known I region antigen, and thus may point to a new I region determinant. Further studies will be aimed at defining these unexpected cross-reactions.

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Density-dependent phosphorylation of a specific protein in cultured cells

THE phosphorylation–dephosphorylation reactions that regulate the biological activities of many proteins are sensitive to changes in physiological conditions[1]. Hormones regulate the phosphorylation of both membrane-bound[2] and cytosolic proteins[3,4], while the phosphorylation of histone[5] and non-histone chromosomal proteins[6] changes during different phases of the cell cycle. The cellular density of mammalian cells grown in culture can affect biological properties such as cell growth[7], locomotion[8], transport[9] and enzyme activity[10,11]. We have examined the phosphorylation of endogenous proteins in cultured mammalian cells at different cellular densities as a means of determining whether cell–cell interactions might affect protein phosphorylation. We report here that the phosphorylability of a specific protein present in several diverse cell lines exhibits density-dependence.

Cell lines designated Balb 3T3 and SV3T3 originated from the Balb A_{31} and SVT_2 lines, respectively, established by George Todaro (NIH); T3 schwannoma originated from RN_2 lines established by S. E. Pfeiffer (University of Connecticut); AFH, an amelanotic variant of B16 melanoma lines, was obtained from J. J. Sauk Jr (University of Minnesota); the SF human diploid fibroblasts were secondary cultures derived in our laboratory.

AFH melanoma cells, T3 schwannoma and SV3T3 fibroblasts do not exhibit density-dependent inhibition of growth, and grow to high densities ($> 10^7$ cells per 100-mm plate). These cell lines were plated at both low and high densities and extracts were prepared 24–48 h after plating. The extracts were incubated with γ-^{32}P-ATP, and the ^{32}P-labelled proteins were separated by sodium dodecyl sulphate (SDS) polyacrylamide slab gel electrophoresis. A protein of molecular weight 110,000 (determined by comparison with molecular weight standards) was heavily phosphorylated in extracts from cells which had been plated at a high cell density, but was only slightly phosphorylated in extracts from low density cells (Fig. 1*a–c*). The *in vitro* phosphorylation of no other protein consistently changed as a function of increased cell density in the cell survey described here. One possible explanation for the increased phosphorylability of this protein in extracts from high density cells could be an increased concentration of this substrate protein. But when cell extracts are incubated with γ-^{32}P-ATP, only sites available for phosphorylation (that is, which have not been phosphorylated in the intact cell) incorporate ^{32}P. Thus, another explanation could be an altered regulation of the phosphorylation of the protein *in vivo*.

In addition to comparing the phosphorylation of extracts from cells which had been plated initially at high and low densities, we have also examined extracts from cells which were plated at low density and then grown to high density. Once again, the phosphorylation of this single protein was higher in the extracts from high density cells than from the low density cells (Fig. 1*d*). We conclude that the phosphorylability of this protein increases when the density of cells on a plate is high, and does not depend on the experimental manipulations used to achieve high cellular density. We have called this protein density-dependent phosphoprotein (DDP).

The effect of cellular density on DDP phosphorylation was also examined in two normal cell lines which exhibit density-dependent growth inhibition. SF, a line of human diploid fibroblasts which grows to a high density (10^7 cells per 100-mm plate) but then becomes quiescent, also showed a greater extent of DDP phosphorylation at high density than at low density (Fig. 1*e*). Balb 3T3 mouse fibroblasts normally grow to a low saturation density of about 10^6 cells per 100-mm plate. These 3T3 cells showed minimal DDP phosphorylation in extracts from both confluent cultures and sparsely populated cultures (Fig. 1*f*). Serum-stimulated (40 % serum) 3T3 cells,

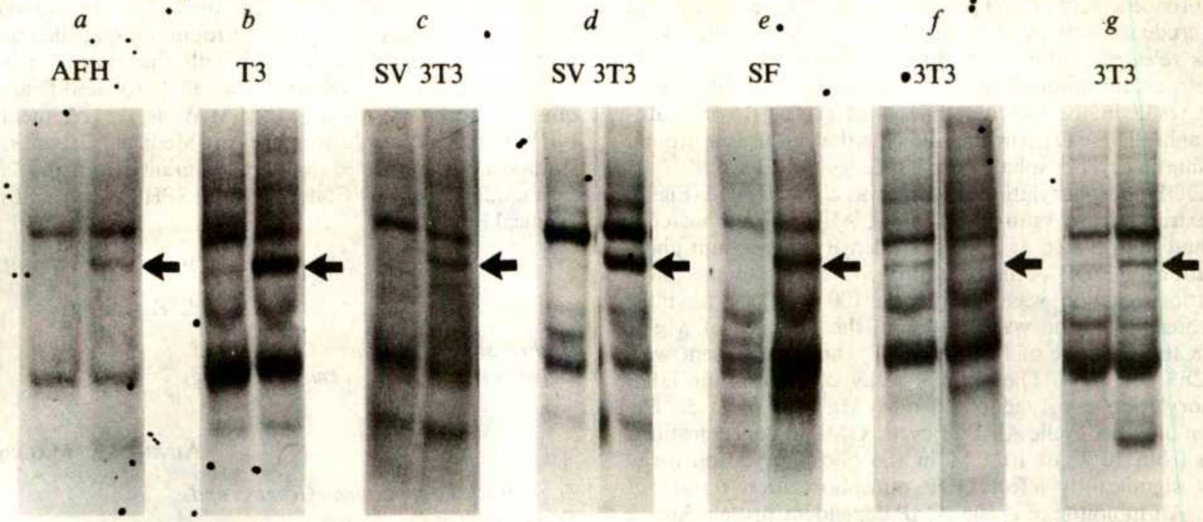

Fig. 1 Autoradiograph of ^{32}P-labelled proteins from mammalian cell lines at low and high density. Various mammalian cell types (*a–c*, *e*) were plated at either low density (10^4–10^5 cells per 100-mm plate) or high density (10^6–10^7 cells per plate) and grown for 24–48 h. The culture media for cells were: DMEM and 10% foetal calf serum for murine SV3T3, Balb 3T3 and human diploid fibroblasts; DMEM and 10% calf serum for rat T3 schwannoma; and RPMI 1640, 10% foetal calf serum and 10% tryptone for murine AFH melanoma. SV3T3 cells (*d*) were plated on 100-mm plates at low density (10^5 cells per plate) and grown to high density (10^6–10^7 cells per plate). Extracts from low density cells were prepared 8 h after plating and those from high density cells 72 h after plating. To obtain sufficient protein from sparse cultures, Balb 3T3 cells were grown in roller bottles. Extracts were prepared from low density cells (before cell contact) 4 d after seeding 100 ml of medium with 2×10^6 cells; high density cell extracts were prepared 8 d after seeding (*f*). In other experiments, Balb 3T3 cells were first grown to confluence (10^6 cells per plate) on 100-mm plates; medium was then removed and replaced with DMEM and 40% foetal calf serum, and growth continued for 48 h until a high density (4×10^6 cells per plate) was reached. Extracts were prepared from low density cells and from these high density cells (*g*). In all experiments, cells were grown at 37 °C and exposed to a humidified atmosphere of 5% CO_2. Samples were prepared by rinsing cells with 0.32 M sucrose, and disrupting the cells by sonication. Protein concentrations were determined by the method of Lowry *et al.*[12]. The crude sonicates were assayed for endogenous phosphorylation as described by Malkinson *et al.*[13]. The 100-μl reaction mixture contained 70–90 μg of protein, 50 mM (*N*-morpholino) ethanesulphonic acid (MES) *p*H 6.5, 10 mM $MgCl_2$, and 1.0–1.5 μM γ-^{32}P-ATP (5-20 Ci mmol^{-1}). After incubation for 3 min at 30 °C the reaction was terminated with an SDS-dithiothreitol 'stop solution', and the tubes were boiled for 2 min. ^{32}P-labelled proteins were separated by SDS–polyacrylamide slab gel electrophoresis[14] and autoradiographs of the dried gels were prepared. Each cell line is represented by a pair of autoradiographs. In each pair, the left autoradiograph was obtained from extracts of low density cells and the right autoradiograph from extracts of high density cells. The arrow indicates the position of DDP on each autoradiograph.

however, grew to densities of 4×10^6 cells per 100-mm plate, and more DDP phosphorylation occurred using extracts from these high density cells than with extracts from sparse plates (Fig. 1*g*). These experiments with growth-regulated human and mouse fibroblasts indicate that increased DDP phosphorylability also occurs in normal cells after growth to a sufficiently high cellular density.

The following experiment illustrates the reversibility of this density-dependent phosphorylation. SV3T3 cells were plated at a low density (10^5 cells per 100-mm plate). At various times during growth, the plates were scored for cell contact, the number of cells per plate was determined, and cell extracts containing equal amounts of protein were phosphorylated (Fig. 2). DDP phosphorylation was minimal when cells were sparse (before cell contact). When 100% of the cells had established cell contact (10^6 cells per plate), DDP phosphorylation began to increase. When the highest density was reached (10^7 cells per plate), DDP phosphorylation was 8–15 times higher than in sparsely populated plates. When these high density cells were diluted and replated at a low density, the absence of cell–cell contacts was associated with a decreased phosphorylability of DDP. When the cell number exceeded 10^6 cells per plate, DDP phosphorylation again increased.

When endogenously phosphorylated proteins from high density AFH or SV3T3 cells were incubated with pronase (30 μg ml^{-1}) at 30 °C for 15 min before the addition of SDS, DDP and all other phosphorylated bands were absent from the autoradiographs. Neither RNase nor phospholipase C had any effect on the phosphorylated bands. Boiling the homogenates before incubation with γ-^{32}P-ATP also abolished all bands from the autoradiographs. Incubation of endogenously phosphorylated proteins with a partially purified preparation of liver phosphoprotein phosphatase (supplied by J. S. Bishop)

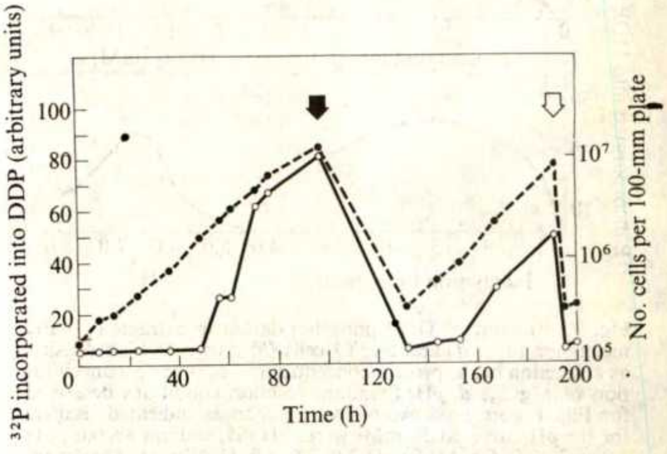

Fig. 2 Amount of DDP phosphorylation in extracts from SV3T3 cells grown to different cell densities. SV3T3 cells were plated at 10^5 cells per 100-mm plate and the medium was changed at 72 h. At the indicated times, cells were removed with 0.05% trypsin and counted. This treatment with trypsin did not affect DDP phosphorylation. Duplicate plates were prepared and assayed for endogenous phosphorylation as described, using 67 μg of protein per assay. When cells had grown to a density of 10^7 cells per plate (indicated by closed arrow), the cells were removed with trypsin, diluted in fresh medium, replated at 10^5 cells per plate and grown again to a high density. At 8×10^6 cells per plate (indicated by open arrow) cells were removed, diluted and replated at 3×10^5 cells per plate. At various intervals the cell number was determined, and extracts were phosphorylated. The amount of ^{32}P incorporated into DDP was determined by scanning the autoradiographs with an Ortec 4310 microdensitometer. The peak height of the optical density is expressed as arbitrary units of ^{32}P incorporated[15]. Cell number (●) and DDP phosphorylation (○) are shown.

reduced DDP phosphorylation on the autoradiograph. When a trichloroacetic acid (TCA) precipitate of ^{32}P-labelled proteins from a crude sonicate was incubated at 37 °C with dilute alkali, ^{32}P was released within 15 h and no phosphorylated bands were seen on an autoradiograph. Incubation with dilute acid did not result in the loss of ^{32}P-labelled proteins from autoradiographs. These experiments indicate that DDP is a protein containing an acyl phosphate ester bond.

The DDP phosphorylation reaction was characterised (Fig. 3) using extracts from both SV3T3 and AFH melanoma cells which had been plated at a high cell density. Maximum phosphorylation of DDP occurred within 3–5 min, and the phosphorylation reaction was linear from 100 to 1,000 μg ml^{-1} DDP phosphorylation was greatest in the presence of Mg^{2+}, slight in the presence of Mn^{2+} or Co^{2+}, and was absent with either Zn^{2+} or Ca^{2+}. The optimal assay conditions for DDP phosphorylation consisted of 10 mM Mg^{2+} at *p*H 6.5. The inclusion of either cyclic AMP or cyclic GMP at concentrations ranging from 10^{-11} to 10^{-4} M in the phosphorylation assay did not significantly affect DDP phosphorylation (data not shown). An inhibitor of cyclic AMP-dependent protein kinase enzymes, isolated from rabbit skeletal muscle[16], caused no inhibition of DDP phosphorylation. Thus, in our assay conditions, the kinase catalysing DDP phosphorylation is independent of cyclic nucleotides. Homogenates from high density cells were fractionated by differential centrifugation into soluble and particulate fractions and the endogenous phosphorylation assay was performed with each subcellular fraction. DDP phosphorylation was observed only with the 100,000*g* supernatant, suggesting that neither DDP nor the kinase enzyme which catalyses its phosphorylation are tightly membrane-bound.

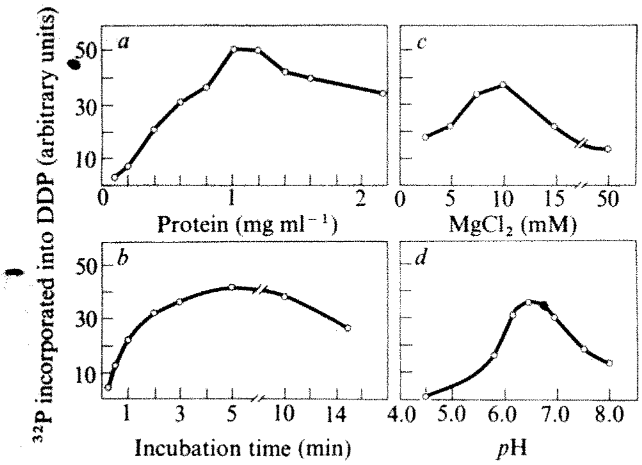

Fig. 3 Amount of DDP phosphorylation in extracts of AFH melanoma (*a*, *c*, *d*) and SV3T3 cells (*b*) grown to high density, as a function of: *a*, protein concentration; *b*, time; *c*, concentration of $MgCl_2$; *d*, *p*H. Standard reaction conditions described for Fig. 1 were used except for the changes indicated. Buffers for the *p*H curve, at 50 mM, were: *p*H 4.5, sodium acetate; *p*H 5.8, 6.2, 6.5, 6.8, MES; *p*H 7.0, 7.5, 8.0, HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethane-sulphonic acid.

Differences in phosphate metabolism have been found between 3T3 and SV3T3 cells, and between high and low density suspensions of 3T3 cells[9,17]. It is unlikely that increased DDP phosphorylation at high density is related to these changes in phosphate metabolism, for DDP is the only protein whose phosphorylation is consistently affected by density. There are several enzymes[18] and contractile proteins[19] of a monomer molecular weight corresponding to DDP, but it is premature to speculate on the identity of this protein. It is interesting that a phosphorylated protein corresponding in molecular weight to DDP has been observed in foetal rat tissues, but not in adult tissues (A.M.M. and C. E. McSwigan, in preparation). In the cellular slime mould *Dictyostelium discoideum*, the number of freely phosphorylable sites per cell decreased at the onset of cell aggregation[20]. Because many membrane properties change as a function of cellular density[21], the biological activity of DDP may eventually be found among the contractile proteins or enzymes associated with the plasma membrane.

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Connectivity of the monoamine-containing neurones in central nervous system of leech

THE functional properties of neurones which contain biogenic amines are usually proposed from the results of studies involving ablation or pharmacological intervention[1]. Monoamine (MA) neurones are seldom identifiable *in vivo*, which impedes direct investigations of their functions. The central nervous ganglia of leeches are comprised of about 350 neurones, nine of which contain amines as their putative neurotransmitters. These MA neurones are vitally and selectively stained by Neutral Red dye[2], a procedure potentially facilitating intracellular studies of this population of biochemically related cells. We report here the electrophysiological properties of the red-staining neurones within the central nervous system (CNS) of the leech.

Chains of five to eight segmental ganglia were dissected from horse leeches, *Haemopis marmorata* (Say), and their MA cells were stained by 0.01% (w/v) Neutral Red in leech saline[3] for about 30 min. Nine widely scattered neurones stain within the three anterior-most ganglia, while seven stain within each of the 18 posterior ganglia (Fig. 1). These neurones included the two Retzius cells (RZ, 50–65 μm), two pairs of lateral cells, one pair ventral (VL), the other dorsal (DL), and a posterior, unpaired medial cell (M). An extra pair of MA cells (E) was seen within each of the three anterior ganglia. These seven small (15–20 μm) intraganglionic neurones shared histochemical properties with the RZ (refs 4, 5), which contain the indolealkylamine, serotonin (5-hydroxytryptamine, 5-HT)[6]. The interconnected 21 segmental ganglia innervate the body by means of paired lateral roots. A stainable neurosoma is situated in each anterior root, and this pair of anterior root cells (AR) probably contains the catecholamine, dopamine[2]. The large RZ send axons to the periphery[4,7], while the small MA cells appear to restrict their connections to the CNS.

Stained cells were impaled with glass micropipettes (4 M potassium acetate) with tip resistances of 20–50 MΩ. Membrane potentials and impulse activities were controlled with currents injected through the recording electrodes by means of balanced bridge circuits. Potentials were recorded on tape (FM, HP 3960) and permanent records were usually produced by playback at ¼ speed into an oscillographic recorder (HP 7404). Such speed reduction avoids attenuation by the oscillograph of the high frequency components of the action potentials.

Stained RZ cells have resting potentials (40–60 mV) and non-invading action potentials (30–55 mV) indistinguishable from those in unstained preparations[2]. Furthermore, the stained pair of RZ remain coupled by an efficacious electrotonic junction. The small MA cells have small action potentials (usually 5–15 mV) which do not actively invade their somata. We recorded simultaneously from a Retzius cell and each of the small ganglionic MA neurones, and representative results are shown in Figs 2 and 4. The impaled MA neurones usually have tonic impulse activities and post-synaptic potentials, and frequently produce bursts of impulses. These bursts usually occur with slowly rising depolarisations of 5–15 mV. As Fig. 2*a* shows, the bursts in the RZ and the small cell usually occur in temporal register, suggesting that they receive a common excitatory input[8]. Cross correlograms of the time intervals between the depolarisations of the MA cell pairs support the suggested pattern of a common excitor. We see this pattern of nearly synchronous depolarisations and impulse bursts between RZ and both DL and M (Fig. 2*a*) as well as VL and E (not shown here). To map more completely the responses of the MA cell population, we impaled and recorded simultaneously from three pairs of small, bilaterally homologous cells—VL–VL, DL–DL and E–E. As Fig. 2*b* (VL–VL) shows, these cell pairs also undergo nearly synchronous depolarisations which generate impulse bursts. We have recorded from pairs of non-homologous small cells and found a similar pattern of common excitatory bursts by the following pairs: VL–DL, VL–M, E–M and VL–E (Fig. 2*c*).

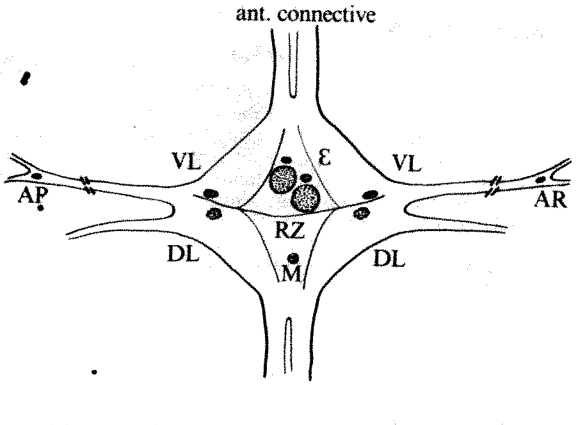

Fig. 1 Diagram of the ventral aspect of a leech segmental ganglion showing the positions of the MA-containing neurones which are stained by Neutral Red dye.

Thus the entire population of MA neurones shares an excitatory input and often fires synchronous bursts of impulses. The addition of 20 mM Mg^{2+}, which blocks chemical synaptic transmission in leeches[9], abolishes these depolarisations and bursts. In view of this finding, we initiated pharmacological experiments on the chemical synaptic nature of the excitatory depolarisations. Serotonin (10^{-5} M) neither enhances nor induces bursts by MA neurones; 5-HT (the putative transmitter of these cells) hyperpolarises RZ, abolishing all spontaneous impulse activity. Acetylcholine (ACh) is a candidate transmitter for it depolarises and excites RZ into impulse activity[10]. Atropine is a competitive inhibitor of ACh at muscarinic receptor sites and it effectively blocks the slow depolarisations and impulses bursts by RZ (Fig. 3). The depolarised bursts return after the atropine is washed from the preparation. Furthermore, the anticholinesterase eserine

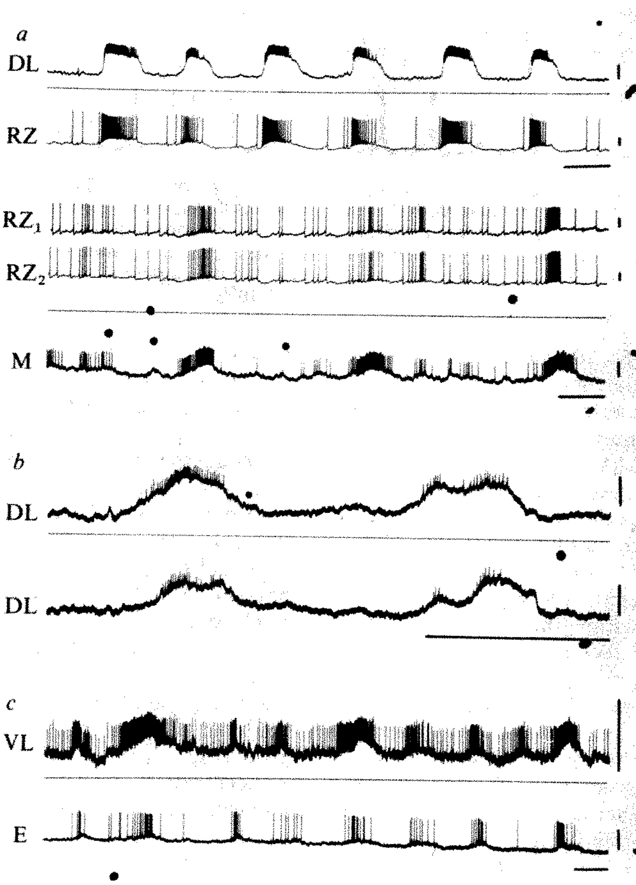

Fig. 2 Intracellular recordings of the spontaneous activity in the red-stained MA neurones of the leech *Haemopis*. Calibrations: 10 mV, 5 s. *a*, Top panel showing the slowly rising depolarisations and the resulting impulse bursts in DL (top trace) and RZ. The lower panel shows three simultaneous recordings from the pair of RZ and an M cell. The RZ maintain perfect impulse synchrony during burst and interburst periods. M depolarises, generating bursts at similar times to the RZ; however, M impulses do not match those of RZ in a 1 : 1 fashion. *b*, Slow depolarisations of both DL cells generate impulse bursts. The membranes of both cells show similar slow potential changes, but the action potentials are not 1 : 1. *c*, Recordings of the depolarisations and action potential bursts in VL (top trace) and E. In this record the impulse frequency of VL is higher than that of E. The magnitudes of the depolarisations appear to differ because of the different gains of each trace.

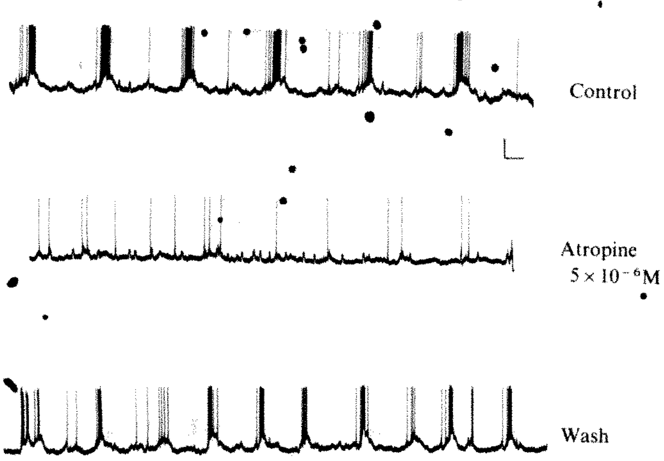

Fig. 3 RZ activity in a stained, bursting preparation. The top panel is a control with depolarisations and bursts at a period of 20–25 s. Atropine blocks the slow depolarisations, but not other unitary excitatory postsynaptic potentials (middle). After washing (bottom), depolarising bursts return but at a period of about 12 s. Calibrations: 10 mV, 5 s.

(5×10^{-4} M) increases both the amplitude and duration of the spontaneous depolarisations seen in the RZ. These eserine-enhanced depolarisations can also be blocked by addition of atropine. We always saw bursts in the small MA cells whenever depolarisations occurred in RZ, and as these depolarisations seem to be mediated by a common excitor, most pharmacological experiments were done with the larger, more accessible RZ. We have verified the effects of atropine on both VL and M cells. Therefore, the central synaptic input which depolarises the MA neurones within the leech ganglion is probably cholinergic.

We passed currents into RZ while monitoring the effects on small MA cells in order to determine the nature of any interconnections among the aminergic population. Inward current directly hyperpolarises RZ and also hyperpolarises the small cells to a lesser degree (Fig. 4*a*). These d.c. potential shifts persist in a high concentration of Mg^{2+}, and increase monotonically with increasing currents. In one case each nA into an RZ produced a potential change of 0.38 mV in DL. Thus the RZ is coupled to each of these small cells by an ohmic junction: M and E (Fig. 4) and VL and DL (not shown). In addition, hyperpolarisation of each of these small cells will also produce a d.c. hyperpolarisation of the RZ. The RZ are coupled to one another by a low resistance pathway such that an impulse in one cell produces an electrotonic potential in the other which exceeds impulse threshold, and therefore the RZ maintain impulse synchrony (Fig. 2*a*). The small MA cells are coupled to the RZ by a higher resistance junction. Each RZ impulse can be detected within the small cell somata as an electrotonic potential of about 0.5 mV. These potentials are resistant to Mg^{2+} and have a latency of less than 1 ms. In high Mg^{2+}, a current-induced burst of RZ impulses will electrotonically depolarise a small MA cell to impulse threshold. Experiments on the homologous, small cell pairs show electrotonic coupling between E–E, DL–DL and VL–VL (Fig. 4*b*). Similarly electrotonic coupling is seen between the following pairs of small, non-homologous cells: VL–DL, VL–M and E–M (Fig. 4*c*). Hyperpolarisations are usually more effective than are depolarisations for all these MA cell junctions.

Thus all nine monoamine-containing neurones within the leech ganglion seem to be electrotonically intercoupled. Electrotonic interconnections provide a route by which positive feedback can generate bursts of impulses[11]. Such bursts are produced by the injection of currents into these MA neurones; however, anodal break stimulation (cessation of hyperpolarisation) is more effective in generating a self-sustaining burst than is depolarisation (Fig. 4*a* and *c*). These anodal break bursts can be induced both in preparations showing no spontaneous bursts, and out-of-phase with the timing of the excitor in those showing spontaneous synchronous bursting. Occasionally we can generate an anodal break burst by the MA neurones in high Mg^{2+} saline. Thus the electrotonic coupling among the MA cells is at least in part responsible for burst formation.

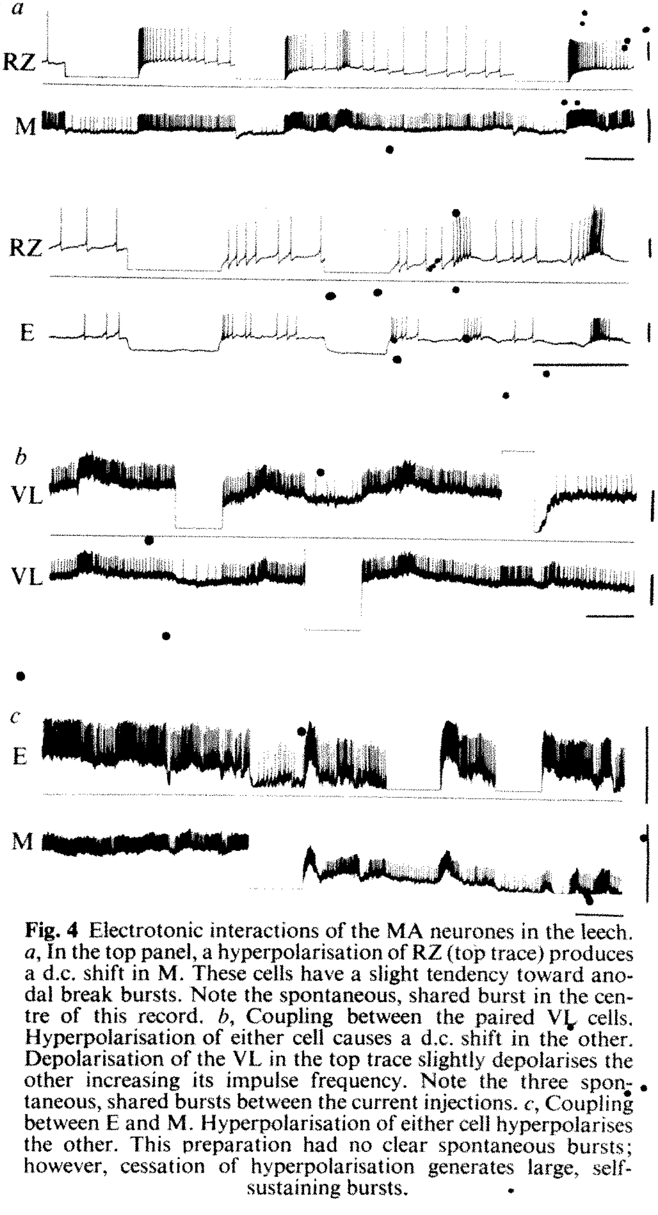

Fig. 4 Electrotonic interactions of the MA neurones in the leech. *a*, In the top panel, a hyperpolarisation of RZ (top trace) produces a d.c. shift in M. These cells have a slight tendency toward anodal break bursts. Note the spontaneous, shared burst in the centre of this record. *b*, Coupling between the paired VL cells. Hyperpolarisation of either cell causes a d.c. shift in the other. Depolarisation of the VL in the top trace slightly depolarises the other increasing its impulse frequency. Note the three spontaneous, shared bursts between the current injections. *c*, Coupling between E and M. Hyperpolarisation of either cell hyperpolarises the other. This preparation had no clear spontaneous bursts; however, cessation of hyperpolarisation generates large, self-sustaining bursts.

The intraganglionic MA cells share a common cholinergic input, and are electrotonically intercoupled. Either of these patterns of connectivity can produce bursts by the MA cells. Presumably the presence of both patterns assures a concerted impulse activity. We do not know why the monoamine-containing neurones burst in concert as their functional role is unknown, except for a peripheral function for RZ in controlling the secretion of mucus from the skin of the leech[12]. Thus, the functioning aminergic neurones in the leech are both identifiable and experimentally accessible.

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Post-mortem changes implicate adenine nucleotides and pyridoxal-5′-phosphate in regulation of brain glutamate decarboxylase

GLUTAMATE decarboxylase (GAD) is the enzyme responsible for the synthesis of γ-aminobutyric acid (GABA), an important neurotransmitter in the CNS (ref. 1). Alterations in GAD activity have been implicated in epilepsy, drug-induced convulsions and neurological diseases such as Huntington's chorea and parkinsonism[2–5]. Although this enzyme has been under study for over 20 yr, little is known of the molecular mechanisms by which its activity is regulated. Recent results suggest that the degree of saturation of GAD by its cofactor, pyridoxal-5′-phosphate (pyridoxal-P), *in vivo* could be determined by a balance between the rate of dissociation of the cofactor from the enzyme brought about by glutamate and the rate of association of the cofactor with the apoenzyme, which is inhibited strongly by the nucleotides ATP and ADP (ref. 6). Thus, it seemed likely that degree of saturation of GAD by pyridoxal-P *in vivo* might be low and might increase as a consequence of post-mortem changes in the levels of ATP and ADP. Additional activation *in vitro* during preparative steps might also occur because nucleotide levels would be reduced during homogenisation. Data presented here confirm these expectations and implicate the adenine nucleotides and pyridoxal-P in the regulation of GABA synthesis.

The percentage saturation of GAD by pyridoxal-P is defined here as the activity of GAD measured in the absence of added pyridoxal-P as a percentage of the activity measured in the presence of saturating levels of the cofactor (100 μM). This approach is possible because the cofactor is tightly bound to the active site in the absence of glutamate and cannot be removed by either dialysis or Sephadex column chromatography[6,7]. As it has been shown that there is a time-dependent inactivation of the enzyme in the absence of pyridoxal-P and in the presence of high concentrations of glutamate, GAD was incubated for only 5 min with 1 mM glutamate. We estimate that the loss in activity is no more than about 5% in the absence of pyridoxal-P in these conditions. When ATP was added to the homogenate to prevent the association of pyridoxal-P with GAD, we used a concentration of ATP fivefold greater than the concentration of ATP required for maximal inhibition of activation by low levels of pyridoxal-P in order to overcome loss of ATP by hydrolysis[6].

If brain extracts were prepared in the conventional manner by decapitating the animal, dissecting the brain at room temperature and homogenising in the absence of ATP, we found that the enzyme was 84% saturated by pyridoxal-P (Table 1). If ATP was added to the homogenising medium, however, the degree of saturation fell significantly to 60% ($P < 0.001$). The difference between these values (25% saturation) represents the *in vitro* activation of the enzyme. Similar significant decreases in the percentage saturation were also observed when ATP was included during the homogenisation of brain samples obtained by freeze-blowing[8] or from heads decapitated into liquid nitrogen (Table 1). To determine whether this *in vitro* activation could be attributed to the binding of endogenous cofactor to the apoenzyme, the concentration of the endogenous pyridoxal-P was directly measured in $HClO_4$ homogenates using tyrosin apodecarboxylase[9]. The results confirmed that there was sufficient pyridoxal-P to account for this activation (0.5–1.0 μM). Further evidence of an *in vitro* activation of the enzyme was provided by the observation that the degree of saturation of the enzyme was significantly lower (65%, $P < 0.01$) when 2% rather than 10% homogenates (ratio of tissue weight to total volume) of conventionally dissected brains were prepared (without ATP). Thus, there seems to be a significant increase in the degree of saturation of the enzyme by pyridoxal-P during preparative procedures *in vitro*.

Table 1 Effects of methods of killing and conditions of homogenisation on the percentage saturation of brain GAD

Method	GAD activity (nmol per mg protein per 5 min) Homogenised without ATP No pyridoxal-P	100 µM pyridoxal-P	Saturation (%)	*n*	Homogenised with 5 mM ATP No pyridoxal-P	100 µM pyridoxal-P	Saturation (%)	*n*
a, Decapitated and dissected at room temperature	6.3±0.2	7.5±0.2	84±5	4	5.0±0.2‡	8.3±0.4	60±2	4
b, Decapitated into liquid nitrogen	6.1±0.4	7.9±0.2	77±3	4	3.2±0.4	7.3±0.5	43±3	5
c, Freeze-blown	4.0±0.3*	6.7±0.2†	59±2	3	2.6±0.1	7.5±0.3§	35±2	4

Saturation was determined by dividing activity obtained in the absence of added pyridoxal-P by total activity obtained in the presence of saturating pyridoxal-P (100 µM). Whole brain from male Sprague–Dawley rats (200–250 g) was removed by three different methods. In (*a*) rats were decapitated and the brains were dissected out at room temperature. Each was then cut along the midline and half was homogenised in about 9 volumes of 5 mM ATP (*p*H 6.5); the rest was homogenised in a similar volume of ice-cold deaerated distilled H_2O. Homogenisation was performed within 2 min of decapitation and samples were immediately centrifuged at 16,000*g* for 30 min at 4 °C. The supernatant was removed and diluted 1:1 with buffer to give 100 mM imidazole-acetate, *p*H 6.5, and 5 mM mercaptoethanol. Two ml of each diluted supernatant was then passed over a column of Sephadex G-25 (bed volume 70 ml) at a flow rate of approximately 2 ml per min to remove ATP, endogenous glutamate, pyridoxal-P, and other low molecular weight compounds. The column had been pre-equilibrated with buffer at the same concentration and kept in the cold. Five ml of column effluent was collected and used for analysis of GAD activity both in the presence and absence of pyridoxal-P (100 µM). The procedure used for the enzymatic assay was similar to that previously reported[17] except that the enzyme was incubated for only 5 min and with only 1 mM glutamate. We have previously shown a one-to-one correspondence in the production of CO_2 and GABA from U-^{14}C-glutamate[7]. In (*b*) rats were decapitated and the heads were frozen in liquid N_2. After dissection at 0–2 °C each brain was ground to a fine powder and divided into two equal fractions for homogenisation and further treatment as presented in method (*a*). In (*c*), brain samples were rapidly removed by freeze-blowing, each was ground to a fine powder and divided into two equal fractions for homogenisation and further treatment as for (*a*). In all cases samples with saturating pyridoxal-P (100 µM) were significantly different from paired samples without pyridoxal-P ($P < 0.02$). Comparing corresponding samples analysed in the absence of pyridoxal-P, significantly lower values were obtained in all cases when the samples were homogenised in 5 mM ATP ($P < 0.01$). A similar comparison of samples analysed with saturating pyridoxal-P showed a significant difference only in the freeze-blow method ($P < 0.05$). All values are mean ±s.e.m.

*Significantly different from similarly treated samples in (*a*) and (*b*), ($P < 0.02$).

†Significantly different from similarly treated samples in (*a*), ($P < 0.05$).

‡Significantly different from similarly treated samples in (*a*) and (*b*) ($P < 0.01$).

§Significantly different from similarly treated samples in (*a*), ($P < 0.05$).

Post-mortem activation of GAD in the intact brain can be seen by comparing the results obtained with ATP-treated homogenates of brains collected by freeze-blowing and the results obtained with similar homogenates of brains dissected at room temperature (Table 1). The freeze-blowing technique removes brain samples and freezes them to liquid nitrogen temperatures within 1 s thereby preventing post-mortem changes in the levels of adenine nucleotides and other energy metabolites[8]. We found that the percentage saturation of the enzyme in homogenates of brain dissected at room temperature (60%) was significantly greater than the percentage saturation in homogenates of freeze-blown brains (34%, $P < 0.001$). The difference between these values (26%) is the post-mortem activation in the intact brain. The activation resulted in an almost twofold increase in the amount of holoenzyme present in the homogenates as indicated by the enzyme activity measured in the absence of pyridoxal-P. Decapitation into liquid nitrogen also reduces the post-mortem activation as shown by the finding that the percentage saturation in ATP-treated homogenates from animals in this group (43%) was significantly lower than the value obtained with similar homogenates from brains dissected at room temperature ($P < 0.001$). A significant post-mortem activation was also observed with samples homogenised in the absence of ATP. In this case, the degree of saturation of the enzyme in brains dissected at room temperature (84%) was significantly greater than the degree of saturation in samples from freeze-blown brains (59%, $P < 0.001$). Since freeze-blowing prevents the rapid post-mortem hydrolysis of ATP which accompanies anoxia[8], the relatively low degree of saturation in these freeze-blown samples could be attributed to the presence of sufficient endogenous ATP which reduces substantially the amount of binding of pyridoxal-P to the apoenzyme. A similar low degree of saturation was not observed in samples from animals decapitated into liquid nitrogen. It has been shown, however, that marked post-mortem changes in the levels of brain metabolites including a 40% reduction in the brain level of ATP occur during freezing by this method[8]. Thus, there is apparently an insufficient amount of endogenous nucleotides present in these homogenates to prevent the activation of GAD.

Our results demonstrate that brain GAD undergoes both a post-mortem activation by pyridoxal-P in the intact brain and a further activation *in vitro* during preparative procedures. The high degree of saturation (84%) observed in homogenates of brains dissected at room temperature are attributable to these effects. When both types of activation were minimised by homogenising freeze-blown brain samples in 5 mM ATP, the enzyme was only 35% saturated. Clearly, this represents an average for many brain regions and compartments and the actual degree of saturation in any particular compartment may differ significantly from this value. The results do indicate, however, that brain GAD is, in general, only partially saturated with pyridoxal-P. Because of the two types of activation reported here and the glutamate-promoted, time-dependent inactivation of the enzyme[17], previously reported estimations of the degree of saturation of the enzyme by pyridoxal-P *in vivo* may not reflect the actual values but instead may largely be the result of the experimental conditions. These same considerations also suggest that studies dealing with alterations in the relationship between pyridoxal-P and GAD in drug-induced or pathological states may need to be reconsidered.

It has been demonstrated repeatedly that brain GABA levels increase rapidly after death[10–13]. The post-mortem activation of GAD in the intact brain described here may be a factor contributing to the post-mortem change in GABA levels.

The post-mortem activation of GAD together with the known effects of adenine nucleotides and inorganic phosphate on the interaction between pyridoxal-P and GAD (refs 6, 14), support the idea that these compounds are involved in the mechanism controlling the rate of GABA synthesis. Although whole brain levels of ATP are quite constant under normal conditions, it is conceivable that fluctuations in ATP levels may occur in various compartments such as presynaptic endings during periods of altered physiological activity[15]. Kennedy *et al.*[16] provided strong evidence that changes in the functional state of nervous tissue result in changes in energy metabolism. Such changes might alter the degree of saturation of GAD by pyridoxal-P and thereby mediate changes in the rate of GABA synthesis. On the other hand, the interactions between GAD, pyridoxal-P, and the adenine or other nucleotides may merely set the general level of activity of the enzyme and regulation may be accomplished by other, as yet unknown, factors.

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Effects of dietary choline on nicotinic acetylcholine receptors in brain

THE administration of choline by injection[1] or diet[2] increases the concentration of acetylcholine (ACh) in the brain. Choline also increases the activity of tyrosine hydroxylase[3] (TOH) in dopaminergic neurones of the caudate nucleus that receive cholinergic inputs. This response can be blocked by atropine, indicating that it is due to the post-synaptic action of ACh. The post-synaptic alteration of TOH activity suggests that the level of ACh also regulates the concentration of ACh receptors (AChR). To produce large differences in brain ACh, we have studied two groups of rats, one deprived of choline and the other loaded with choline. We found high concentrations of ACh in the brain associated with increased amounts of nicotinic AChR.

Male, Sprague–Dawley rats, 60 d old (230–300 g) from five litters were assigned to either choline-deficient or choline groups using the split-litter technique. Choline-deficient animals were maintained *ad libitum* on a diet lacking choline (ICN Biochemicals) and were given distilled water to drink. The choline groups were maintained on the same diet but choline chloride 15 mg ml^{-1} (Sigma) was added to the distilled water in the drinking bottle. All animals were housed in groups of four to a cage. After 21 d, they were decapitated. The brain and hind leg muscles from each animal were processed individually to extract and quantify the receptors present. All procedures were carried out at 4 °C. Each individual tissue was homogenised in ten volumes of 0.32 M sucrose and 10 mM sodium phosphate, *p*H 7.4. The homogenate was centrifuged at 100,000*g* for 60 min. The pellet was resuspended (ten volumes of original weight) in 10 mM sodium phosphate, *p*H 7.4 and 1% Triton X-100 and shaken gently for 105 min. The homogenate was then centrifuged at 100,000*g* for 60 min. The resulting supernatants (S_2) of brain and muscle were assayed for AChR using ^{125}I-α-bungarotoxin which binds to these receptors with unparalleled affinity and specificity[4]. The nicotinic AChR from brain are similar to the AChR from muscle and electric fish in that they are located at the synapse and have similar toxin binding kinetics, solubility, isoelectric point and hydrodynamic properties[5–10]. Radioactive toxin was prepared by the solid phase lactoperoxidase method yielding a monoiodinated product (specific activity 1.2×10^4 Ci mol^{-1}) and the assay system was based on the use of small columns of CM-50 (Pharmacia) to retain excess ^{125}I-bungarotoxin while allowing receptor–toxin complexes to pass through[11]. This is the opposite principle to the DEAE filter disk assay[12]. All assays were carried out in duplicate and parallel samples were pre-incubated with a 100-fold excess of unlabelled toxin. Specific binding was taken to be the difference between the two assays with and without isotope dilution by unlabelled toxin. There was a 26% increase in the concentration of specifically bound ^{125}I-α-bungarotoxin in the brains of animals which were maintained on the choline diet when computed in proportion to protein extracted ($P < 0.001$) or wet weight ($P < 0.01$). The absolute amount of non-specific toxin binding was less than 25% in all cases and did not differ significantly between groups. No significant differences were observed in the case of AChR from muscle. The increase in brain AChR was not due to malnutrition or a non-specific effect on growth, for brain protein and brain weight were not significantly different between the two groups. The daily intake of choline by the choline group was approximately 250 mg per animal. The body weight gain throughout these experiments was not significantly different between the two groups. (See Tables 1 and 2).

Additional rats were maintained in the same conditions and the brains were sectioned into cerebral hemispheres (including olfactory bulbs), remaining forebrain, brain stem and cerebellum, to determine whether there were regional differences in the relationship of AChR to the dietary manipulation. Significant differences in the density of AChR were observed in the cerebral hemispheres (48% increase in the high choline group) and forebrain (43% increase) thus accounting for most of the whole brain differences. A slight but insignificant increase was found in the brain stem. In agreement with other reports, only minimal amounts of AChR were found in the cerebellum[13–15]. Brain weights and extracted protein did not differ between the groups for any brain section.

Thus, these studies suggest that the receptor concentration is positively related to ACh level. Because the range of choline consumption used in the two groups was within the range of variation that could occur in the human diet[2], we suggest that alteration in diet can alter brain function by controlling not only the amount of ACh released but also the density of receptors with which it may interact. This observed correlation between transmitter level and receptor density has significant implications for the relationship between cholinergic neurones and behaviour. Indeed it has been reported that a patient with tardive dyskinesia has been successfully treated with large doses of choline[16]. Although little is known about the development and functional implications of AChR in brain, ACh has long been implicated in the physiological basis of several forms of behaviour[17], including memory and learning. We are currently investigating the relationship between diet-induced changes in AChR and learning in the developing and adult rat. The possibility of altering such behaviour by dietary manipulation has profound implications.

Table 1 Effects of choline and choline-deficient diets on brain weight, body weight, whole brain protein and nicotinic AchR concentration in whole brain and muscle

Group	Brain weight (g)	Body weight (g)	Total brain protein mg (S_2 supernatant)	pmol toxin per g brain wet weight	pmol toxin per mg brain protein	pmol toxin per g muscle wet weight	pmol toxin per mg muscle protein
Choline	2.07±0.02	116±6.9	114±2.0	3.29±0.13*	0.056±0.001†	0.3549±0.04	0.0152±0.001
Deficient	2.06±0.02	128±4.7	120±5.1	2.61±0.12	0.042±0.001	0.3359±0.04	0.0138±0.002

Values are expressed as mean ±s.e.m. of 11 animals. Aliquots of supernatant were incubated with ^{125}I-α-bungarotoxin for 60 min at room temperature and then assayed. Radioactivity was determined with a Nucleus γ scintillation counter at an efficiency of 50%. Protein was determined by the method of Lowry *et al.*[18] using bovine serum albumin as a standard.

* $P < 0.01$, Student's *t* test.

† $P < 0.001$, Student's *t* test.

Table 2 Effects of choline and choline-deficient diets on brain weight, brain protein and nicotinic acetylcholine receptor concentration in brain sections

Group	Section	Brain weight (g)	Brain protein (mg) (S_2 supernatant)	pmol toxin per g wet weight	pmol toxin per mg brain protein
Choline	Cerebral hemispheres	1.24±0.05	76±4.8	4.32±0.56	0.067±0.004*
Deficient		1.23±0.04	84±11.6	3.23±0.43	0.045±0.004
Choline	Remaining forebrain	0.33±0.03	14.06±1.23	0.3804±0.02*	0.071±0.004†
Deficient		0.27±0.04	13.13±2.06	0.2424±0.02	0.050±0.004
Choline	Brain stem	0.20±0.02	6.96±0.88	0.2217±0.02	0.060±0.008
Deficient		0.25±0.04	9.46±2.9	0.2181±0.03	0.055±0.004
Choline	Cerebellum	0.29±0.01	15.34±0.83	0.0339±0.01	0.008±0.002
Deficient		0.30±0.01	14.26±1.3	0.0382±0.01	0.007±0.002

Values are expressed as mean ±s.e.m. of three or four animals.

* $P < 0.01$, Student's *t* test.

† $P < 0.001$, Student's *t* test.

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Electrophysiological distinctions between the taste and smell of amino acids in catfish

VERTEBRATES possess two principal systems, olfaction and taste, adapted to respond to peripheral chemical excitation. Olfaction in terrestrial vertebrates is the more acute chemosensory system, having lower thresholds to volatile stimuli than the gustatory thresholds to nonvolatile compounds[1,2]; thus, olfaction was termed the 'distance' sense and gustation the 'contact' sense. With fishes, however, chemical stimuli are present in the aqueous medium and it is difficult to distinguish between olfactory and gustatory stimuli or to explain the development of two chemical senses in the primitive aquatic vertebrates. Fishes exhibit important morphological distinctions between olfactory and gustatory systems both at the periphery and centrally[3], and yet, in catfish, amino acids are the most effective stimuli known for both systems[4,5]. This sensory overlap does not reflect a simple duplication of receptor function, however, as the olfactory and gustatory systems of catfish differ in amino acid sensitivity, specificity and response characteristics, which could allow for the functional separation of the chemical senses in an aquatic environment.

Channel catfish, *Ictalurus punctatus*, approximately 70–200 g, were immobilised with Flaxedil (gallamine triethiodide; 0.1 mg per 100 g body wt) and positioned in a Plexiglas container with aerated well-water containing MS 222 (tricainemethane sulphonate; 1:8,000 dilution) perfusing the gills throughout the experiments; this combination of Flaxedil and MS 222 provided for a more stable preparation than either agent alone. Stimuli dissolved in well-water, approximately *p*H 7.8, were introduced into a continuous flow of well-water directed to the maxillary barbel or olfactory capsule, and dilutions were determined by photodensitometry of dye solutions. Taste solutions were diluted at least to 60%, and olfactory stimuli at least to 25% of their applied concentrations. For the taste experiments, the eye was removed and a branch of cranial nerve VII (facial) to the maxillary barbel was exposed and placed on a Pt–Ir wire electrode and covered with mineral oil to prevent drying. To record olfactory receptor responses, a flap of tissue dorsal to the olfactory capsule was removed and a metal-filled glass capillary electrode tip plated with Pt-black[6] with a ball diameter between 15 and 25 μM was positioned between adjacent olfactory lamellae near the midline. Both gustatory and olfactory electrical activity were amplified by a conventional capacity-coupled preamplifier, passed through an averaging circuit (Sanborn Model 350–1400 amplifier operated on the a.c. linear mode) with a rise time of 1.0 s and displayed on a pen recorder. The underwater electro-olfactogram (EOG) (ref. 7), an olfactory mucosal slow potential[8], was recorded with calomel electrodes via Ringer-agar filled capillary pipettes, amplified by a direct-coupled amplifier and displayed on a pen recorder. An essential requirement for successful recording preparations was the use of freshly obtained fish. A rapid decline of olfactory and gustatory receptor sensitivity occurred with increased holding time in the laboratory[9].

Catfish olfactory and gustatory responses revealed many similarities, but were functionally distinguishable by four criteria: phasic–tonic response characteristics, amino acid thresholds, neural spike synchrony and ordering of effective amino acids. The asynchronous neural response of the maxillary

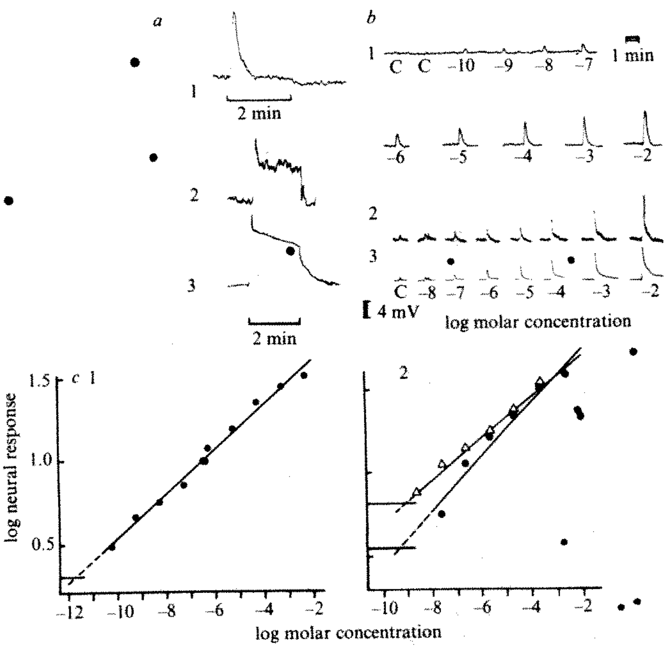

Fig. 1 *a*, Catfish taste and smell responses to a 2-min stimulation with 0.1 mM L-alanine; summated taste recording exhibits only a phasic response (1), while summated olfactory neural (2) and EOG (3) responses show both phasic and tonic components. *b*, Responses to applied concentrations of L-alanine (taste) and L-cysteine (smell); neural taste (1) and simultaneously recorded olfactory neural (2) and EOG (3) responses to applied stimulus concentrations are shown. Lack of olfactory tonic component due to short stimulus duration. *c*, Power relations[19] between chemoreceptor responses and amino acid stimulus concentrations. (1) Taste neural responses; $\log R = 0.132 \log c + 1.84$, $r = 0.99$. (2) Olfactory neural (▲) and EOG (●) responses; $\log NR = 0.125 \log c + 1.95$, $r = 0.99$; $\log \mathrm{EOG} = 0.163 \log c + 2.06$, $r = 0.98$. Taste and olfactory neural responses exhibit similar slopes (range 0.07–0.14); EOG slope is somewhat larger (range 0.14–0.29); dotted lines show interesection of linear regression lines with control responses yielding threshold values. Taste is more sensitive than smell in catfish for the compounds tested. All plotted values have been corrected for dilution and are estimates of the stimulus concentrations at the receptors; taste solutions were diluted by 0.2 log unit and olfactory solutions were diluted by 0.6 log unit as determined by photodensitometry of dye solutions. The olfactory neural response to 10^{-2} M L-cysh (b2) was omitted from the graph (*c*2) due to partial synchronisation of the neural spike activity[11,12]. The processing of this partially synchronous activity by the amplifier averaging circuit resulted in the larger than expected response amplitude, and was therefore not used in determining the olfactory neural threshold.

barbel taste nerve to an amino acid stimulus revealed a quickly adapting phasic response (Fig. 1, *a*1) with thresholds averaging $10^{-11\pm1.7}$ M (Fig. 1, *b*1, *c*1) for the more effective amino acids. The absence of the tonic component of the gustatory response was due possibly to adaptation occurring at the synapse between the taste bud cells and the innervating axon[10].

Neural and EOG recordings from olfactory neurons, neuroepithelial cells which do not form synaptic connections until the receptor cell axons reach the central nervous system in the olfactory bulb, exhibited a phasic component followed by a tonic response which remained throughout the stimulus duration (Figs 2, 3). Olfactory thresholds were less than those observed for the taste system and averaged $10^{-8.4\pm1.0}$ M (Fig. 1, *b*2, *c*2). Also, a partial synchronisation of the axonal spike activity[11] occurred with the more responsive preparations when long interstimulus intervals (> 5 min) and high concentrations ($\geqslant 10^{-4}$ M) of the more potent amino acids were utilised. Self stimulation of the olfactory organ by the EOG has been a suggested mechanism for the neural synchrony[12]. Gustatory neural activity, however, always remained asynchronous.

The difference in olfactory and gustatory thresholds is not simply a reflection of the differences in the neural recording techniques. The results with the Pt-black ball method (olfactory neural recording), which samples a smaller population of neurons than the Pt–Ir electrode (gustatory recording), are consistent with those obtained from the EOG recordings, which measure the voltage change due to the current flow through the extracellular resistance of the olfactory epithelium from all the receptors being stimulated[8].

Chemoreceptive specificity of both systems was determined with several amino acids and their derivatives. Both olfactory and gustatory responses were strongly influenced by the position of the amino group. Amino acids having an amino group in the α position (those derived from proteins) were the most effective. Olfactory and gustatory receptors were also able to discriminate enantiomeric pairs, indicating that the chemoreceptors involved possess a corresponding chirality. The L-isomer of an amino acid was always more effective than its D-form, although certain D-amino acids were more stimulatory than other L-forms. A major difference, however, between olfactory and gustatory responses to amino acids in the catfish involved the amino acid side chain. Although both chemosensory systems of catfish were able to detect extremely dilute concentrations of amino acids, each system was specific for particular side groups of the amino acids. The more effective amino acid stimuli based on the neural responses of both chemical systems are listed in Fig. 2; however, neither list correlates with the amino acids essential for growth in this species[13].

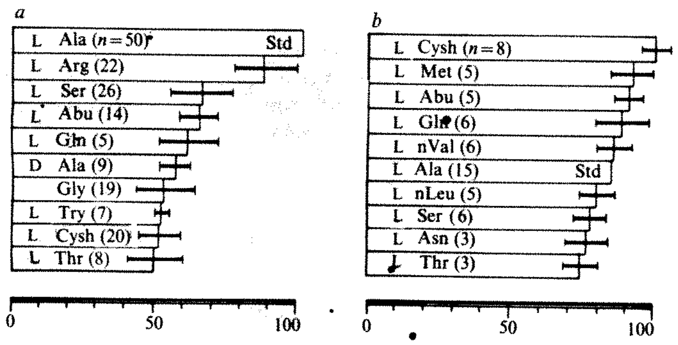

Fig. 2 Comparison of stimulatory effectiveness of the ten most potent amino acids tested on the maxillary barbel and olfactory mucosa at 0.1 mM applied concentrations; estimated concentrations at the receptors were 6×10^{-5} M for the taste (*a*) and 2.5×10^{-5} M for the olfactory (*b*) stimuli as determined by photodensitometry of dye solutions. Relative position of each chemical is represented as a percentage of the response to the standard (Std), 0.1 mM L-alanine. *n*, number of fish tested. Bars indicate s.d.

The taste system was highly responsive to the amino acids of low molecular weight having unbranched and uncharged side chains. A major exception was the response to L-arginine, the second most effective taste stimulus, which is a positively charged, higher molecular weight amino acid. The ability of an amino acid to initiate a large gustatory response fell off rapidly after arginine, and the tenth compound in Fig. 2, L-threonine, elicited a response only 50% of that of the most effective chemical, L-alanine, tested at equal stimulus concentrations.

The olfactory system was not as specific as the taste system and was responsive to a group of amino acids. The sulphur-containing amino acids, L-cysteine and L-methionine, were most effective, with less of a decrease in effectiveness as one proceeds down the order in comparison with taste. The tenth compound, L-threonine, produced a response of approximately 75% of that of the most effective olfactory stimulus tested at equal molar concentration. In general, EOG recordings reflected a similar listing of effective amino acid stimuli, but the order was not identical with that for the neural recordings.

Amino acids are likely to be important in various aspects of fish behaviour. Both cartilaginous and bony fishes orient towards an amino acid source[14–16]; however, the relative importance of olfaction and taste in the detection of the amino acids stimulus and in the initiation of specific behavioral responses is generally unknown. In catfish, conspecific chemical communication by pheromones is thought to be mediated entirely by the olfactory sense[17], and feeding behaviour primarily by the gustatory sense[18]. If certain amino acids or combinations of amino acids provide chemosensory information concerning social or feeding behaviour, then a difference between the olfactory and gustatory receptor responses could code for the appropriate behavioural responses. Although the olfactory and gustatory receptor mechanisms are not understood, each system may have more receptor sites for particular amino acids physiologically relevant to that system.

Whether amino acids such as alanine and arginine, the most effective taste stimuli tested, signal a natural food source in the animal's environment, or whether compounds such as cysteine and methionine, the most efficacious olfactory stimuli tested, are of pheromonal nature, is yet to be determined.

The present experiments indicate that although the olfactory and gustatory systems of the channel catfish are highly sensitive to the α-amino acids, each system responds differently to its chemical environment as determined by electrophysiological recordings.

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Calcium utilisation during anaerobiosis induced by osmotic shock in a bivalve mollusc

DURING aerial exposure, some littoral marine bivalve molluscs, for example *Mytilus*[1] and *Mercenaria*[2,3], have been shown to close their valves and respire anaerobically. The major by-product of anaerobiosis in the clam *Mercenaria mercenaria* is succinic acid which is neutralised by the dissolution of calcium carbonate previously deposited in the shell[2,3]. The ability of bivalves to respire anaerobically has been well documented, an early review on this subject[4] records a variety of species which are able to survive long periods in the absence of oxygen. Most evidence for anaerobic metabolism in bivalves is based on observations that on return to aerobic conditions, oxygen consumption rapidly rises to levels above normal[5,6]. A similar 'over shoot' in heart rate has been reported[1,7,8]. These observations have been explained as representing an increase in circulatory and respiratory activity associated with the repayment of an 'oxygen debt' incurred during the period of anaerobiosis. The changeover from aerobic to anaerobic respiration in bivalves has been reported to occur when the oxygen tension of the surrounding water falls to very low levels or to low tide when the bivalves may close the valves[7,9,10]. We report here results which are part of an extensive study of osmotic and behavioural responses of *Scrobicularia plana* (Da Costa), a euryhaline estuarine species, to varying concentrations of seawater and demonstrate the use of calcium from the shell to buffer the products of anaerobiosis. The concentrations of sodium, potassium, magnesium and calcium were measured by atomic absorption spectrophotometry in the body fluids and medium under different conditions of osmotic stress at 10 °C.

When *Scrobicularia* were transferred from aerated 30‰ seawater to aerated 6‰ seawater, the concentrations of Na^+ and other ions, except Ca^{2+}, in mantle cavity fluid and blood fell gradually to the level of the external medium within 14 d (Fig. 1). At first the valves remained closed for long periods but after 7 d pumping activity took place for more than 50% of the time. In contrast to the other ions, the calcium concentration in the blood rose to a maximum value of about 30 mM (about three times the normal concentration in 30‰ seawater) within 5–7 d of the transfer, after which time the concentrations of calcium in all body fluids dropped towards that of the external medium (Fig. 2*a*). From day 18, a low stable level of calcium was maintained comparable with previously published values for other bivalves. Indeed the rise in calcium concentration has not been reported previously because former studies have determined calcium concentrations after a long acclimation period of 3–4 weeks (refs 11–13). The *p*H of the extra-pallial and mantle cavity fluids changes in a manner similar to calcium (Fig. 2*b*).

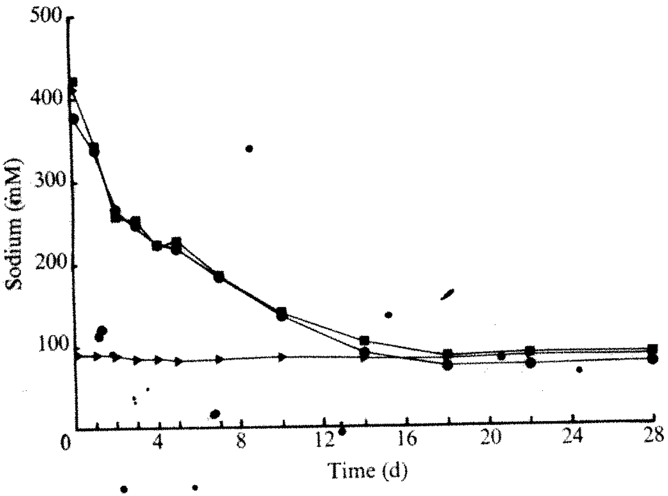

Fig. 1 Na^+ concentrations in *S. plana* transferred directly from 30‰ seawater (day 0) to 6‰ sea water in well aerated conditions (■), mantle cavity fluid; (●), blood from the ventricle; (▲), medium. Each point is the mean of four determinations made on samples pooled from 12–14 individual *S. plana* selected randomly. Throughout the experiment the animals were fed regularly on *Tetraselmis* and the medium was changed every 24 h. Ionic determinations throughout were also made on pericardial fluid and pedal haemolymph with similar results which have been omitted for clarity.

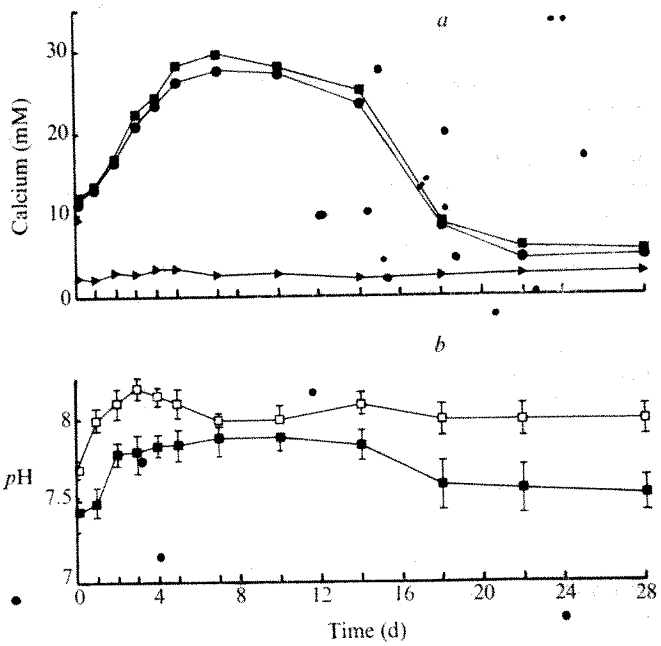

Fig. 2 *a*, Ca^{2+} concentrations of *S. plana* transferred directly from 30‰ seawater (day 0) to 6‰ seawater. Procedure and symbols as in Fig. 1. *b*, *p*H of mantle cavity fluid, medium (symbols as in Fig. 1) and extrapallial fluid (□) from same animals as (*a*). Extrapallial fluid obtained by drilling the right valve between the adductor muscles. Bars represent s.d. of 10 samples from each of 10 animals. Determinations by Ingold micro *p*H assembly with Radiometer, M63 *p*H meter.

The increase in calcium concentration over the first 7 d is correlated with our observations of the behaviour of *S. plana* under these extreme changes in osmotic concentration. Recordings of valve movements and gill pumping activity show that after reduction of the salinity of the medium, animals largely remain closed but gradually expose the mantle tissue and from the seventh day begin to interact more with the medium, the increase of valve movements and pumping activity coinciding with the fall in calcium concentration and *p*H. During the initial period when the valves of *Scrobicularia* are closed, we have shown by microdiffusion and thin layer chromatography that ammonia accumulates in the mantle cavity and that succinic acid is the major organic acid accumulated in the tissues. This has been confirmed by De Zwaan (personal communication).

To determine whether the rise in calcium is due to anaerobic metabolism during the acclimation period, *Scrobicularia* were placed in 30‰ oxygen-free seawater under nitrogen. The calcium levels in all body-fluids rose slightly to 14–16 mM and remained at that level over the 6-d period (Fig. 3*a*, A). During these 6 d the valves remained open. On the sixth day (Fig. 3a, B), the bivalves were osmotically stressed by changing the medium to 6‰ oxygen-free seawater, resulting in valve closure and inactivity. The concentration of calcium increased reaching a maximum of about 30 mM after which it fell towards that of the medium (Fig. 3a, C). The *p*H value of the mantle cavity fluid (over the first 6-d period) is lower than that of the medium, as in *Scrobicularia* respiring aerobically in normal seawater (Fig. 3b), but upon transfer to 6‰ oxygen-free seawater, the *p*H values correspond with those of Fig. 2b. On the 13th day some *S. plana* were closed forcibly by counteracting the hinge ligament with rubber bands and within 3 d the calcium concentrations in the body fluids of these animals increased by 50% (Fig. 3a, D). In *Scrobicularia* kept in 30‰ oxygen-free seawater with their valves forcibly closed, there is a rapid increase in the calcium concentration over a 2-d period to about the same level as those reached by animals in 7 d when subjected to an osmotic shock under aerobic conditions (Fig. 4). After the second day, the valves were allowed to gape normally and the calcium concentrations dropped to normal levels within 2 d. Unlike animals subjected to osmotic shock, those in oxygen-free normal seawater kept their valves open and continued pumping activity. We suggest that continuous water flow through the mantle cavity may explain the lack of a noticeable rise in calcium concentration, for in these conditions no anaerobic metabolites will accumulate and in consequence no buffering will be required.

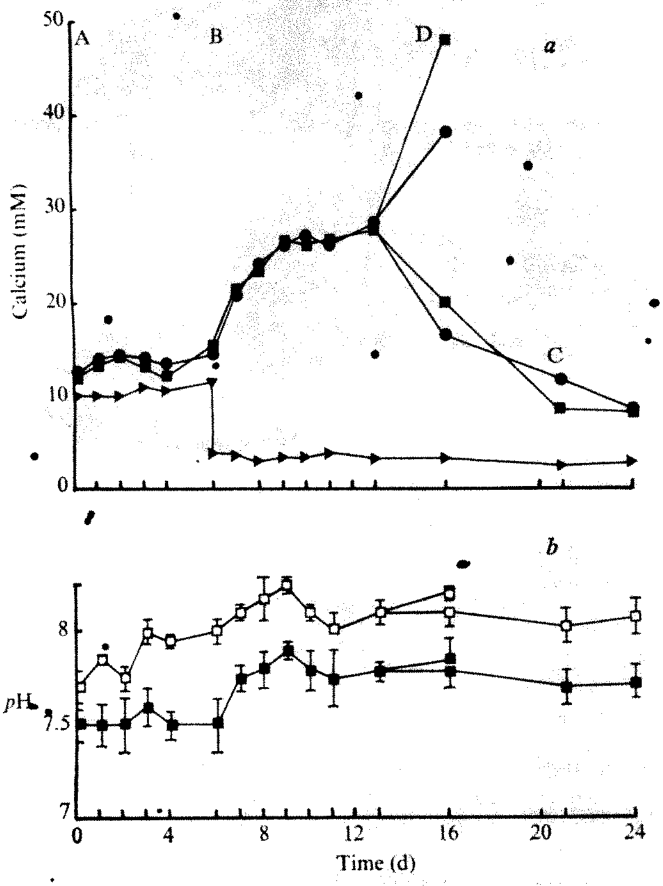

Fig. 3 *a*, Ca^{2+} concentrations of *S. plana* placed in oxygen-free 30‰ seawater on day 0 (A). (Symbols and procedure as in Fig. 1.) After 6 d the animals were additionally subjected to osmotic stress, by immersion in oxygen-free 6‰ seawater (B and C). After a further 7 d the valves of 20 animals were forcibly closed (D). *b*, The *p*H values in animals under the same conditions as Fig. 3 *a*, determined as in Fig. 2.

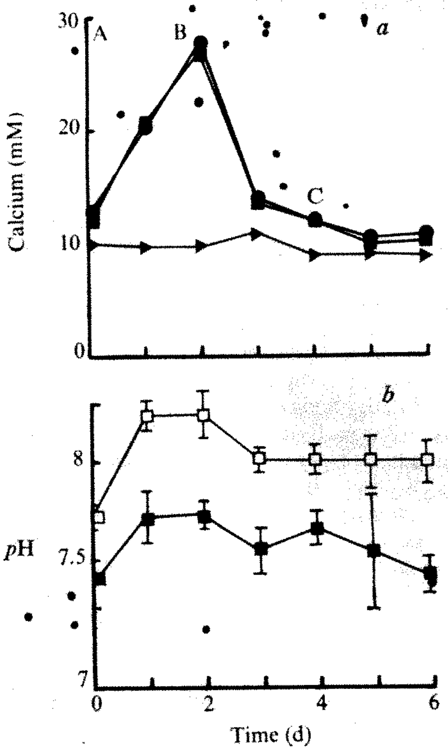

Fig. 4 *a*, Ca^{2+} concentrations and *b*, *p*H values of *S. plana* with valves forcibly closed transferred from aerated 30‰ seawater to oxygen-free seawater (A). After 2 d the bivalves were allowed to gape normally while remaining in the same medium (B). After a further 2 d the medium (30‰ seawater) was aerated (C). Samples and analytical procedure as in Figs 1 and 2.

Adverse environmental conditions, such as osmotic stress, may cause *S. plana* to close its valves for periods of up to 7 d. During prolonged valve closure respiration is anaerobic and, due to lack of ventilation of the mantle cavity, metabolites, for example succinic acid and ammonia, accumulate. A parallel increase in calcium ion concentration, probably by dissolution of the shell, is thought to act as a means of buffering these metabolites during an initial period of acclimatisation to the extreme salinity change.

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X-ray microanalysis of freeze-dried ultrathin frozen sections of a regulating epithelium from the snail *Otala*

MANY epithelial cells are known to regulate the flow of water and ions across the epithelium. This has been observed in various animals[1] and is probably an essential feature of all living organisms. Hibernating *Otala lactea* (Mollusca, Gastropoda, Pulmonata) can survive long periods of drought without access to water by regulating the evaporative water loss from the mantle epithelium[2]. Machin[2] has shown that these snails are as efficient as insects at controlling water loss when they are hibernating, yet when they are active and crawling they lose water at the same rate as does a free water surface. To achieve this the animals produce an osmotic gradient across the mantle epithelial cells of approximately 4.5 Osm kg^{-1} from the cell base to the apical plasma membrane[2]. X-ray microanalysis of freeze-dried frozen sections has shown that the levels of silicon, chlorine, potassium, iron and zinc are significantly greater in hibernating (regulating) mantle tissue than in tissues taken from normal actively crawling snails, and a gradient can be observed within these cells.

Hibernating snails were kept in a dry and undisturbed place in the laboratory[2] while others, which were active, were enclosed in a glass tank with lettuce leaves. Small samples (1 mm^3) of mantle tissue were excised from both hibernating and active snails within 20 s of their removal from their containers. The tissue was frozen on to brass specimen holders[5] by immersion into liquid nitrogen slush at −210 °C (ref. 6). Blocks of tissue were carefully trimmed so that the square blockface included a vertical section through the superficial layers of the mantle epithelium. Trimming and sectioning (130 nm) were done in a cryostat containing an ultramicrotome [5,6] at temperatures at which ribbons of ultrathin sections could just be obtained, that is control blocks at −72 °C; regulating at −76 °C. 'Dry' ribbons of sections were pressed into contact with Formvar-coated nickel grids[5–7], freeze dried at their cutting temperatures for 3 h in the cryostat and in the presence of dry nitrogen, warmed slowly to −15 °C overnight and brought to ambient temperature in the presence of dry nitrogen. They were then coated with 20–30 nm of carbon to prevent rehydration[7].

Sections were analysed either in EMMA 4 (analytical electron microscope) equipped with LINK system energy dispersive detector or in the AEICORA analytical microscope (courtesy of Kratos Ltd, AEI Scientific Apparatus). The specimens were held in graphite inserts to reduce the background readings. Analytical conditions were carefully controlled and matched between both instruments—accelerating voltage, 60 kV; beam current as measured by Faraday cage, 4.0 mA; spot size, 100 nm; overall c.p.s., 200–700; counting time 100 s.

Gupta *et al.*[3] have studied the distribution of three ions within an insect Malpighian tubule by analysing hydrated 'thick' frozen sections of 1–2 μm. In our experience there are considerable advantages in the use of freeze-dried ultrathin frozen sections. The morphology of the cells is sufficiently clear to relate the structures seen in ultrathin frozen sections to those seen in sections of conventionally fixed and embedded material. The good resolution in our micrographs is due to the initial thinness of the section and the removal of water by freeze drying. This also means that there is less lateral spread of electrons around the area of the probe and consequently leads to an improvement in analytical resolution with good peak to background ratios.

Analysis of tissue from ribbons of ultrathin sections of consistent thickness makes it possible to express the results by volume (that is $P-b$) rather than by mass (personal communication from H. Höhling). We felt that the results expressed in this way have greater biological significance than relative mass fractions, $((P-b))/W$, where $P-b$ is the integrated peak minus background for an element, and W is the continuum or measurement of mass), provided that sections are of uniform thickness and freeze drying causes no lateral redistribution of labile elements. The continuum measurements for the regulating and control cells showed that there was no significant difference in section thickness (means, where n = at least 30 measurements, regulating 3,382 ± 94; controls 3,387 ± 231). Previous work suggests that there is no significant redistribution of diffusible elements during sectioning or freeze drying, and Gupta *et al.*[3] have claimed that there were no 'parallel changes in either the characteristic counts or the distribution of elements' in their sections before and after drying.

The subcellular detail of the regulating epithelium is shown in the electron micrographs (Fig. 1*a*, *b*, and *c*)[9]. Figure 1*a* shows an ultrathin frozen section and Fig. 1*b* an enlargement of the apical region. The results of the X-ray microanalysis of this and other sections are summarised in diagrammatic form in Fig. 2, and show that in the apical region there is a marked increase in the relative concentrations of both chlorine and potassium, and that there is a significant concentration gradient within the cells of the regulating epithelium from the apical plasma membrane to the nuclear regions at the base of the cells. There is, however, a difference between the 'light' and 'dark' areas of the nuclei: it is not clear at this stage if the 'dark' areas of the nucleus represent the condensed chromatin. There is also a greater variance in the chlorine and potassium data than for the other elements shown in Fig. 2. It may be that this is an indication that these elements are predominantly unbound and that their concentrations are not constant. The lower variances for phosphorus and calcium may be due to the fact that they are at least partially 'bound'.

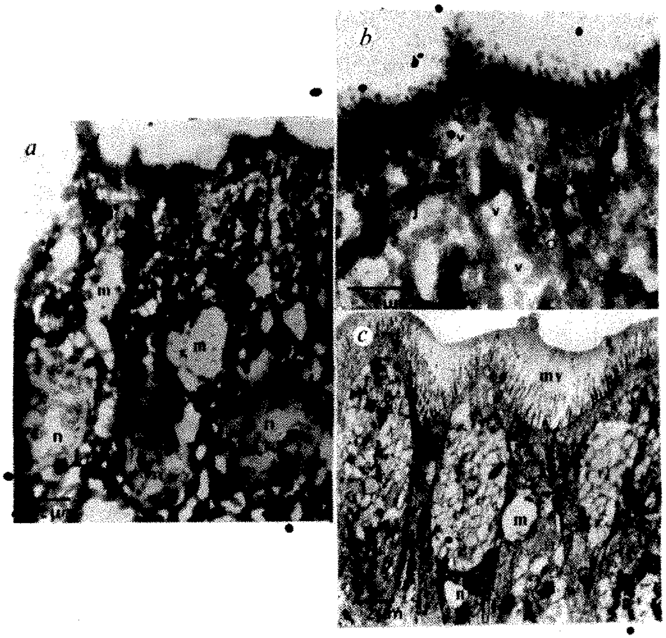

Fig. 1 *a*, Low power electron micrograph of freeze-dried ultrathin frozen section of the regulating epithelium *Otala lactea*. *b*, High power electron micrograph showing the detail in the apical region of the regulating epithelium (from *a*). *c*, Conventionally prepared section of regulating epithelium after fixation and embedding. mv, Microvilli in the apical region; m, mucocyte cells; n, nuclei; j, junctional complexes; v, vesicles (in *b*); lv, (in *c*) laminate vesicles; arrows (*b*) indicate mitochondria; circles (*b*) enclose ice-crystal artefacts.

The silicon levels in the apical region of the regulating epithelium are significantly higher than in the controls (1,011 ± 72 regulating; 685 ± 68 controls). A concentration gradient is established similar to that shown for chlorine and potassium. This cannot be an artefact due to silicon contamination from the glass knives because cutting with diamond knives does not reduce the levels of silicon observed. It cannot be due to contamination from silicon in the oils of the microscope vacuum system because this element is absent from the oils used in our instrument. X-ray microanalysis has also established that there is no silicon in the carbon used to evaporate

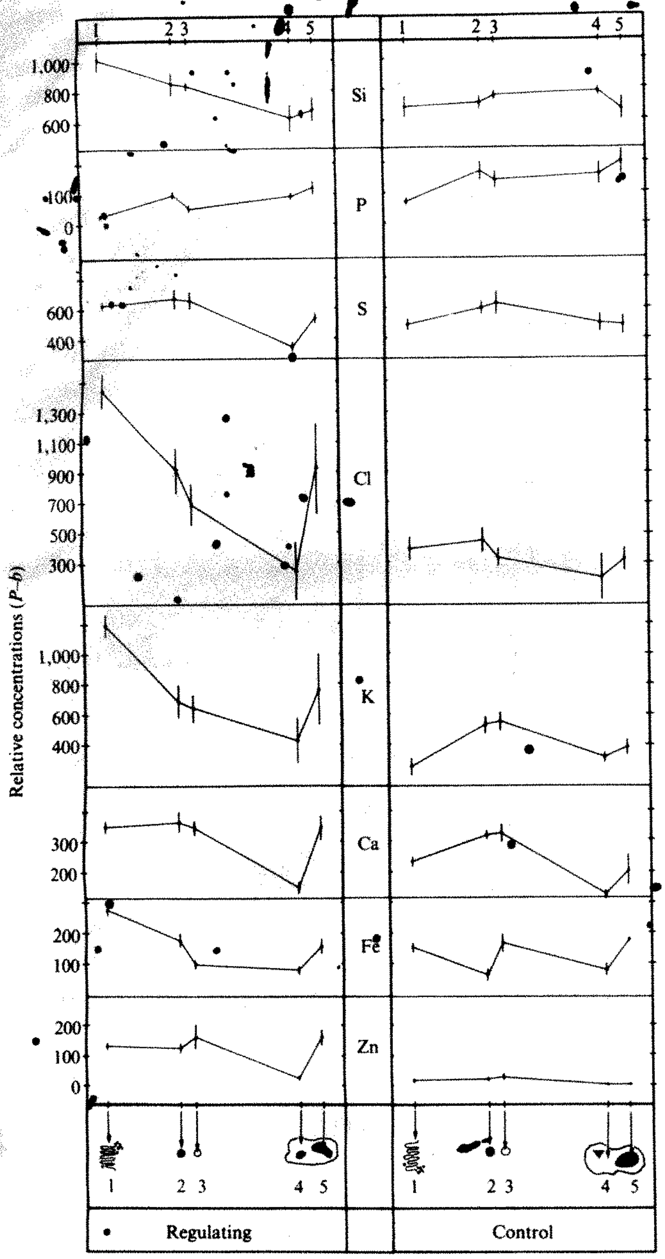

Fig. 2 Elemental composition (by volume) of various elements at four levels with the epithelial cells in regulating and control snails (*Otala lactea*). 1, Apical microvilli region; 2, mitochondria; 3, adjacent ground cytoplasm below each mitochrondria; 4, nucleus 'light areas'; 5, nucleus 'dark areas'. The variances shown are the standard errors of the means and reflect true biological variation rather than errors in acquiring the analytical data. No figures are given for sodium because the sodium levels in general were too low to reflect any significant trends. When potassium levels were high, however, the sodium levels were low. In a few examples the higher sodium levels were always accompanied by lower potassium.

the 20–30-nm layer of carbon over the sections. And it cannot be due to electrons being scattered by the denser organelles, thereby generating silicon X-rays within the lithium-drifted silicon detector, because the mitochondria show a greater electron density but not the highest level of silicon. We feel therefore that silicon may have a greater biological significance than has been assumed.

The changes in concentration of iron and zinc between regulating and control epithelium indicates that these elements are related to fundamental physiological processes within the cells. The increased concentration of zinc, for example, may be related to its role as an enzymatic cofactor associated with carbonic anhydrase, which is known to be present in mantle epithelial tissue[8].

These preliminary results have shown that X-ray microanalysis of freeze-dried ultrathin (130 nm) frozen sections is sufficiently sensitive to detect physiologically significant changes in the concentration of elements at the subcellular level. Further measurements on the elemental composition of structures within these cells are being made and these results will be published in the near future.

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Right-left asymmetry of myelin development in epiretinal portion of rabbit optic nerve

DOMINANCE is the priority or preferential activity of one member of any bilateral pair of structures in the body. The phenomenon of dominant hand, foot and eye for example is well known in humans and this basic sidedness is thought to be cerebral in origin. Based on experiments on audiogenic seizures evidence has been produced for an asymmetry of cerebral function in certain inbred strains of mice[1]. In other animal species dogs are known to have preference of paw, and right–left eye dominance in the visual cortex of cats has been demonstrated[2]. Various theories have emerged to explain ocular dominance[3,4]. It is generally accepted that this is not associated with significant structural differences between the two halves of the brain[5]. Apart from morphological evidence for left–right asymmetrics in temporal lobes of speech regions[6], very little evidence for anatomical differences has been produced in humans or other animals. The rabbit retina is unique in having bundles of myelinated nerve fibres extending equatorially from the optic disk, which are unmyelinated at birth but in which myelination begins at 9–10 days of age[7]. I present here results which indicate that the sequence of myelination is asymmetrical between the right and left eyes, one being dominant.

Rabbits are born with closed eyes which open on the 10th day after birth. I noted that 19 out of 24 rabbits opened the right eye first followed by the left several hours later while the remaining 5 rabbits opened the left eye first.

The optic nerve enters the retina 3–4 mm above the midbrain from the optic head and extends nasally and temporally into two bands of 'medullary rays'. The epiretinal strips were dissected after whole body perfusion with 4% glutaraldehyde[7] and immersed in 1% osmic acid. The eye which opened first showed blackening of the fibres at a comparatively faster rate than the other eye (Fig. 1*a*, *b*). These empiretinal strips were embedded as previously described[7].

Serial 1 µm sections were cut from nasal to temporal side of the epiretinal strips of both right and left eye from 24 animals; 12 more rabbits were also included in this study. The sections from the 9-d-old rabbit showed only a few myelinated nerve fibres close to the disk of one eye, while no myelinated fibres were present in the other (Fig.

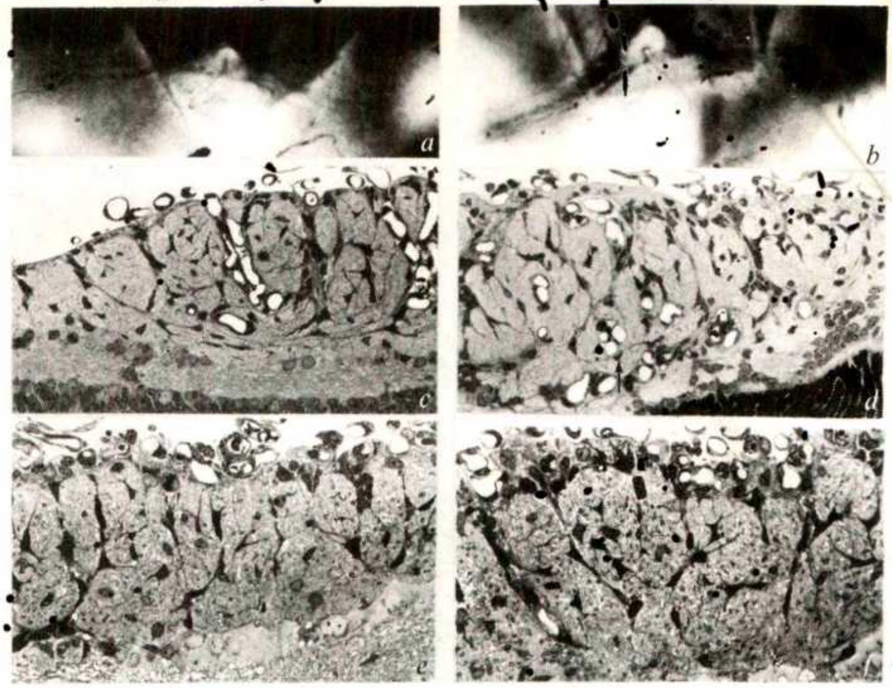

Fig. 1 *a*, *b*, Epiretinal portion dissected from 9-d-old rabbit. Left (*a*) and right (*b*) eye photographed 15 min after osmication. Note the intensity and size difference of the L–R strip. *c*, *d*, 1 μm section from 9-d-old rabbit of left (*c*) and right (*d*) eye epiretinal strip through the optic disk stained with toluidine blue showing a few myelinated fibres in the right strip (*d*) (arrow). ×160. *e*, *f*, Left (*e*) and right (*f*) eye epiretinal strip section through the optic disk of 10-d-old rabbit. Note the dominant myelinated fibres in right (*f*) eye. ×160.

1*c*, *d*). The right eye was usually more advanced than the left.

From the 10th day onward, both eyes show myelinated fibres, but where the right eye opened first, the number of myelinated fibres was much greater than in the left eye which opened several hours later (Fig. 1*e*, *f*). In cases where the left eye opened first the left eye was more advanced in the number of myelinated fibres. This anatomical feature of myelin dominance was difficult to see later than 22 d after birth. The nasal and temporal halves of the strip of each eye are also asymmetrical in both size and development of myelin sheaths, the latter side being dominant.

Examination of 1 μm sections from right and left optic nerves from 1–3-d-old rabbits also showed that earlier myelination of the nerve fibres in the right eye occurred in about 75% of the animals, and earlier myelination of the left was seen in 25%.

In the adult rabbit the anatomical asymmetry which exists during early development could not be observed morphologically. It was, however, demonstrated by light stimulation studies.

It is likely that the earlier onset of myelination in one eye is associated with the dominant brain hemisphere, which during development would most probably show morphological asymmetry, possibly one side (half of body) developing at a comparatively faster rate rather than the size difference betwen the two halves of the hemisphere. The ratio 3 : 1 between the right–left dominance suggests that the mechanism is genetically controlled.

It is tempting to draw a comparison between the asymmetry in rabbit and man in that the cortical dominance may be due to early maturation of one half of the hemisphere and nerve fibres arising from it rather than neurone size.

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New type of representation for mapping chain-folding in protein molecules

Conformational details of small molecules can easily be visualised in the usual stereo diagrams used by crystallographers, but such details cannot be readily visualised for protein molecules made up of thousands of atoms. For such large molecules special types of representations become necessary for depicting their three-dimensional structure, in the form of two-dimensional diagrams. In view of this, many attempts to represent the conformational details in protein molecules, have been reported[1–7]. I present here a new method for mapping the folding of the backbone polypeptide chain of protein molecules. This representation brings out all the features of protein folding, with no degeneracy caused by depicting the conformational details, and is useful in readily recognising all the secondary structures, without ambiguity, as one progresses along the chain.

The backbone conformation of a protein chain can be completely specified by the twin dihedral angles (φ, ψ)[8] since the 'peptide unit' can be taken to be planar for all practical purposes. (There are some indications that the peptide unit is nonplanar[9,11], but the deviations from planarity can be considered to be very small when compared to the full range of variations for the dihedral angles φ around the single bond $N—C^\alpha$ and ψ around $C^\alpha—C$.) It has become customary to plot the (φ, ψ) values at each C^α-atom of a protein molecule in the Ramachandran map to show how the dipeptide conformations conform to the limits of the hard-sphere and soft-sphere models in the (φ, ψ) map[12]. But the details of the secondary structures as one progresses along the chain are not readily available in this representation. In order to achieve this, some single parameters have been developed to be used in chain plots[5,6], but there is an inevitable degeneracy in these chain plots, since many conformations will give rise to the same value for such a single parameter representing a particular residue.

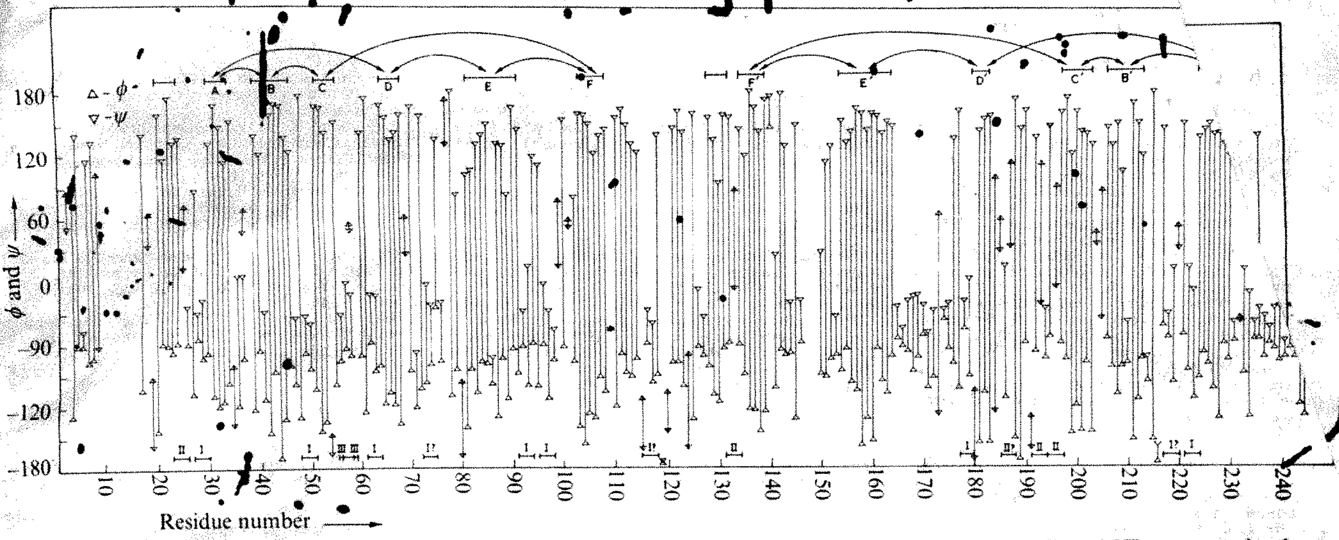

Fig. 1 The new type of mapping for the protein molecule α-chymotrypsin. △, φ values; ▽, ψ values. I, II and III represent the three types of bends. The arrow-headed bows indicate the bonding mates of the β-stretches A, B and so on. For further details see text.

The following chain representation overcomes the difficulties mentioned above, and seems to give a good overall picture of the folding of the protein molecule as one progresses along the chain.

The residue numbers are sequentially taken along the X-axis and the corresponding φ and ψ values are plotted along the ordinate with different symbols. Symbol Δ is chosen for φ and, ∇ for ψ. For proper visual effect these two symbols for a given residue are joined by a vertical line. This kind of chain representation for mapping of protein folding is shown in Fig. 1 for the case of α-chymotrypsin (ref. 13).

In this representation the α-helices are seen as stretches of short vertical lines around −55° on the ordinate (see, for example, residues around 240). The β-stretches are seen as stripes of long vertical lines encompassing almost the whole range of the ordinate (see for example, residues around 225).

The three types of bend conformations[13,14] or loops can be at once recognised and identified in this kind of map. The residues corresponding to the three types of loops are marked as I, II and III at the bottom of Fig. 1; characteristically, loop I has two short lines sandwiched between two long lines (see for example residues 27–30); loop II has a similar pattern, but the third residue has ψ value less than the φ value (see for example residues 23–26). Loop III is simply a part of 3.0_{10}-helix and an example is seen in the residues 55–59. It should be noted that changes from these characteristic patterns would give tell-tale evidence of distortion in the loops. In Fig. 1 such distorted loops have been marked with a question-mark and these agree well with the observations of Birktoft and Blow[13].

Important feature of the folding in α-chymotrypsin are the two approximately similar domains, each having a 'barrel' of anti-parallel β-sheets, termed cylinder I and II by Birktoft and Blow[13]. Each cylinder is made up of six anti-parallel β-stretches, each of which is hydrogen-bonded to two neighbouring β-stretches, and thus forming a barrel. The six β-stretches have been marked by horizontal lines just above the corresponding plots in Fig. 1. They have been labelled A, B, C, D, E, F in cylinder I and A′ B′ C′ D′ E′ F′ in cylinder II, following Balasubramanian[15]. The bonding mates of each of the β-stretches have also been indicated by arrow-head 'bows'. The broad similarity between the patterns in this mapping for cylinder I and II is obvious.

Shotton and Watson[16] found reverse internal homology in tosyl elastase and extended the idea to α-chymotrypsin which belongs to the same family of proteins—the serine proteases. Nishikawa and Ooi[17] also contend that the primary structures of the two similar domains correspond to each other in the opposite sense; that is, residues 29 to 112 correspond to residues 230 to 133 in an approximate way. Hence the β-stretches involved in the cylindrical domains have been appropriately labelled[15], and an approximate mirror symmetry can be seen in the map (Fig. 1), with the mirror situated near residue number 122. But a closer look may justify a translational symmetry too. It is readily seen that for the β-stretch of residues 20–24, there is a corresponding one of residues 128–132 (these sequences of residues can in a way be called β-stretches, though there are no regular hydrogen bonds with a neighbouring extended conformation); these stretches precede the stretches of residues forming the similar domains. However, in the distorted β-stretch B, there is a 'break' at residue 41 (a short vertical line) and correspondingly, there is one at 210 in the distorted β-stretch B′. The paradoxical existence of both the approximate mirror symmetry and translational symmetry in the map arises because there is already an approximate mirror symmetry in the stretches 29–112 corresponding to cylinder I and 133–230 corresponding to cylinder II (a mirror could be 'placed' near residue 60 and another near residue 190). Due to the distortions in the similar domains[13], one cannot resolve the question unequivocally. Nevertheless, this type of mapping is very useful for observing such intrinsic details of chain folding.

The method of mapping chain folding in protein molecules presented here seems to have many advantages over many other types of representations reported in the literature, and can be used as a uniform and stereotyped 'finger printing' for all protein molecules.

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